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Conference Proceedings
Carlisle, Pennsylvania
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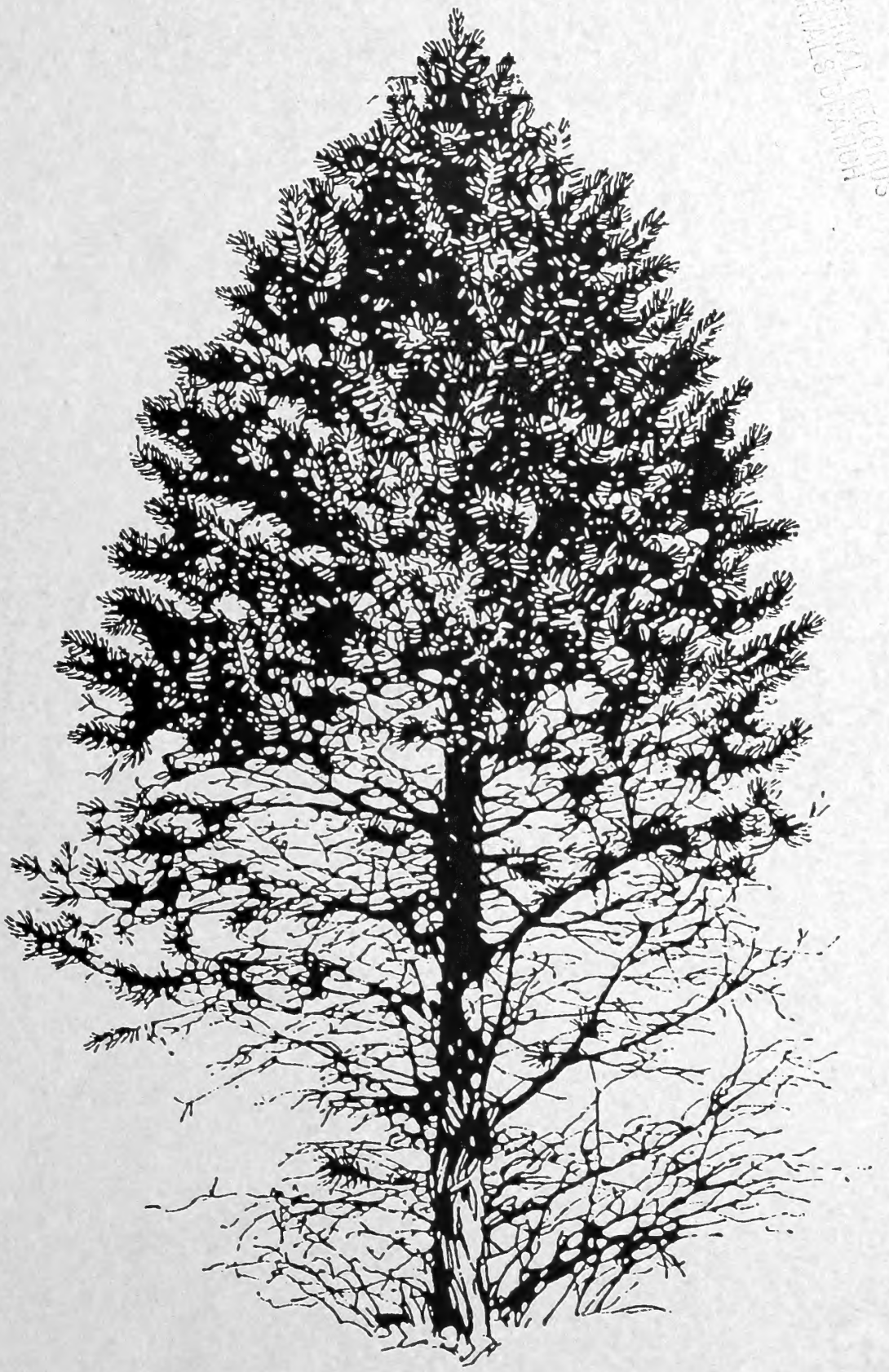

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Results of current research on foliage diseases of conifers and hardwoods in several countries are presented in 29 papers. The results include aspects of disease cycles, infection, host-parasite relations, ecology, epidemiology, genetic resistance, taxonomy and control.

KEY WORDS: Tree diseases, conifers, hardwoods, foliage diseases, needlecasts, needle blights, twig blights, leaf spots, anthracnoses.

This proceedings reports on research with pesticides and other chemicals that have the potential for use in the management of foliage diseases. However, the USDA Forest Service does not endorse or promote the use of materials not registered by the U.S. Environmental Protection Agency under the auspicies of the Federal Insecticide, Fungicide, and Rodenticide Act, as amended. Questions or comments about any of the reported research should be directed to individual authors.

USDA Forest Service
General Technical Report WO-56

RECENT RESEARCH ON FOLIAGE DISEASES
Conference Proceedings

Carlisle, Pennsylvania
May 29 - June 2, 1989

William Merrill and Michael E. Ostry
Editors

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Dedication

Dr. Glenn W. Peterson (retired)

After receiving his doctorate from Iowa State University in 1958, Glenn joined the USDA Forest Service at Lincoln, Nebraska, where he spent the next 30 years of his professional career. He made numerous valuable contributions to the understanding and control of foliage and seedling diseases and diseases of windbreak trees in the Great Plains. He also worked closely with the Department of Plant Pathology of the University of Nebraska. During his career Glenn received several awards from the U.S. Department of Agriculture and the USDA Forest Service, and an Award of Merit from the Great Plains Agricultural Council.

Glenn was active in international forest pathology and served on special assignments in South America and the People's Republic of China. He was involved in the I.U.F.R.O. W. P. S2.06.04 from its inception, chairing it from 1980 to 1985, hosting its third international conference in 1984, and editing the ensuing proceedings volume. We dedicate this volume to him.

FOREWORD

The International Union of Forestry Research Organizations Working Party on Foliage Diseases (formerly Needle Diseases) held a conference on Conifer and Hardwood Foliage Diseases at Dickinson College, Carlisle, Pennsylvania, 29 May-2 June 1989. The conference consisted of presentations of papers, discussions, and demonstrations of computer systems. The papers covered on-going research on disease cycles, infection, epidemiology, host-parasite relations, ecology, disease resistance, impact and losses, and taxonomy and control. There were 27 papers presented, of which 25 were submitted for publication. Four additional papers were submitted for publication but not presented either due to lack of time or inability of the authors to obtain travel funds. Of these 31 papers, 12 were from 9 countries outside of North America. Dr. David Minter, International Mycological Institute, demonstrated a computerized index of IMI needlecast specimens and references, retrievable by pathogen species, genus or family, host species, country, region within country, collector, date, etc. This will be expanded to include all IMI records. Dr. Bruce Nash, The Pennsylvania State University, demonstrated an interactive graphics program to train field survey personnel to accurately estimate the percentage of leaf area affected by various insects or fungal pathogens.

During the conference the attendees toured three commercial Christmas tree farms growing primarily Douglas-fir, Fraser fir and Colorado blue spruce. The growers discussed the problems encountered in producing this speciality crop including: site preparation, stump removal, fertilization and pH control, ground covers, planting, shearing, harvesting, debris management, and control of weeds, insects, and fungal pathogens. Specialized equipment for pesticide application, stump removal, shearing, and tree-digging for the ball-and-burlap ornamentals market was demonstrated.

After the conference most of the non-US attendees traveled to State College, Pennsylvania and spent an additional day in the field where they examined the production of Scots pine Christmas trees on former coal strip mine spoil banks. They also visited one of several research sites along an acid deposition gradient where a large interdisciplinary team from The Pennsylvania State University and The Ohio State University is investigating the effects of acidic deposition on growth of Appalachian hardwoods. They examined growth of several species of hardwoods in various "open-top" chambers where the plants were grown in charcoal- filtered air and protected from rainfall, or exposed to various combinations of ambient air and rainfall. They then examined an 80-year-old plantation of *Picea abies* exhibiting symptoms similar to those of the European "Waldsterben". The attendees also viewed a large, modern coal processing plant which provides coal for export, a large strip-mining operation where approximately 15 meters of over-burden were being removed to reach the coal seam, and finally the poor growth and mortality (caused by various factors, including insects and diseases) of various coniferous and hardwood species planted in attempts to reforest old strip mine spoil banks.

At a very brief business meeting at the end of the conference the members of the Working Party noted the large increase in number and diversity of research papers and that the committee is meeting its objectives and should be continued. They - and visitors to the conference - noted the extensive, wide-ranging and completely open and frank discussions and exchange of ideas. Such frank and open discussions have become almost extinct in most "scientific" meetings but remain a hallmark of IUFRO meetings. In view of the fact that the first two meetings of this Working Party were chaired by Europeans and held in Europe, while the second two were chaired by Americans and held in the U.S.A., the Working Party urges the Directors to appoint someone from East Asia to the vice-chair and eventually chair of this group with the aim of holding a meeting of this group in Asia in the future.

We thank Mrs. Linnea Slaybaugh and Mrs. Brenda Holcomb who entered the papers into the computer, formatted them, and then entered the various editorial changes. Some papers went through five "polishing" editions. However, in most papers the editing changes were minor and the authors are responsible for the accuracy of the statements, as all had the opportunity to review the edited manuscripts. The opinions expressed in these papers may not reflect the policy or the opinions of the Department of Plant Pathology, The Pennsylvania State University, nor those of the U.S. Department of Agriculture.

We owe many thanks to Mr. & Mrs. William Fetherolf, Mr. Marlin Koch, Mr. Burt Bachert, and Mrs. Mary Olson and David Olson for their hospitality in conducting tours of their Christmas tree farms, and to Mr. George Perry, Schuylkill County Cooperative Agricultural Extension Agent, who assisted in organizing and conducting these tours.

I owe special thanks to my colleague, Mrs. Nancy Wenner, who, as co-hostess of the conference, took charge of all local arrangements for the conference, coordinated travel and room arrangements for the attendees, obtained information packets for them, handled registration, and provided a photographic record of portions of the conference.

I also thank Dr. Bruce Nash, Dr. Michael Simini, Dr. Glenn Stanosz, and Ms. Tina Driesbach, without whose help the conference would not have gone nearly as smoothly.

William Merrill, Chairman
IUFRO Working Party S2.06.04, Foliage Diseases

IUFRO W.P. S2.06.04 - Foliage Diseases Working Party History

(taken, in part, from Millar)

The IUFRO Working Party S2.06.04, originally entitled "Needle Diseases", was established in 1971. It met informally in Minneapolis in 1973 during the Second International Congress of Phytopathology. In retrospect, it appears that the original working party was composed primarily of those working on Lophodermium needlecast. The Working Party held its first international conference in 1975 at Schmalenbeck, Federal Republic of Germany. Stephan and Millar edited a proceedings volume (3). The group met again in 1976 at the XVI IUFRO Congress in Norway.

The Working Party held its second international conference in 1980 at Sarajevo, Yugoslavia. Nineteen scientists from ten countries attended the meeting. One day was devoted to Lophodermium and Naemacyclus on pines (nine papers) , but the meeting was broadened to allow a second day to be devoted to other needle diseases (nine papers). Millar edited a proceedings volume (1).

The Working Party held its third international conference in 1984 at Gulfport, Mississippi. Sixteen scientists from five countries attended; another submitted a paper for a total of fifteen papers from six countries. Only two papers dealt with Lophodermium. Peterson "coordinated" a proceedings volume (2).

In 1987 I was questioned by Dr. Dimitri whether it would be feasible to expand the scope of the Working Party to include diseases of broad-leaved trees. While I was in the process of polling the membership, I was informed that the name of the group would henceforth be "Foliage Diseases" and that the membership should be expanded to reflect this change. In actuality, the membership was split nearly evenly on the change. Those interested primarily in ecology or taxonomy favored the expansion; those interested primarily in epidemiology and control were opposed. Opposition centered around the conceptual differences in dealing with long-cycled diseases characterized by tardive epidemics versus short-cycled diseases capable of explosive epidemics.

During my tenure as chairman, I computerized all membership data and purged from the roster over two dozen who had not responded to any letter during the previous eight years. A number of former members also had retired and were removed from the list unless they specifically requested to maintain their liaison with the group, e.g., Drs. Collin Millar and Finn Roll-Hansen. Several new members were added, including the first from the People's Republic of China. The Working Party now is quite lean, but contains 68 scientists actively researching foliage diseases on six continents.

This was the fourth international conference of the Working Party. Twenty-nine scientists from eight countries attended and members from three other countries submitted papers, for a total of twenty-nine contributed papers from eleven countries. Only a single paper dealt, in part, with Lophodermium.

Thus, it can be seen that this Working Party is still evolving from a small group of scientists interested primarily in Lophodermium needlecast to a group with very broad and diverse interests, not only in subject matter but also in approaches to research on foliage diseases. The challenge for the incoming chair and vice-chair will be to obtain greater participation from those studying foliar diseases of broad-leaved plants, as well as from the USSR and countries in the Tropics.

William Merrill, Chairman
IUFRO W.P.S2.06.04, Foliage Diseases 1 Sept 1989

LITERATURE CITED

1. Millar, C.S. (ed.) 1981. Current research on conifer needle diseases. Forestry Dept.,Aberdeen Univ., Aberdeen, Scotland. 113 p. [Aberdeen Univ., Old Aberdeen, AB9 2UU **Scotland** (£ 5)]

2. Peterson, G.W. (tech. coord.) 1985. Recent research on conifer needle diseases. USDA For. Serv. Gen. Tech. Rep. WO-50. 106 p. [U.S.D.A. Forest Service, PO Box 2417, Washington, D.C. 20013. **U.S.A.** (free)]

3. Stephan, B.R., Millar, C.S. (eds.) 1975. Lophodermium an Kiefern. Mitteilungen d. Bundesforschungsanstalt f. Forst- u. Holzwirtschaft, Reinbeck. No. 108. 201 p. [Kommissions-Verlag, Max Wiedebusch 2000, Hamburg 36, Dammstorstrasse 20, **F.R.G.** (DM 17)]

Conference Participants

Front row, from left: N. Wenner (cohostess), T. Dreisbach, S. Livsey; second row: D. Minter, J. Minter, U. Heiniger (W.P. vice-chairwoman), D. Hsiang, C. Savinelli, G. Hudler, W. Merrill (W.P. chairman and cohost), F. Roll-Hansen; third row: R. Stephan, G. Snow, A. Wulf, B. Nash, T.Kubono, H. Roll-Hansen, R. Stack; fourth row: R. Scharpf, G. Chastagner, J. Walla, R. Jalkanen, M. Ostry (cohost), G. Stanosz, S. Redlin, F. Jewell, G. Adams, and Y. Suto. Not pictured: B. Towers and M. Simini.

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CONTENTS

Phacidium Infestans Sensu Lato: Taxonomy, Nomenclature, Distribution and Hosts[1]

Finn Roll-Hansen[2]

Abstract.–*Phacidium infestans* sensu lato includes *P. infestans* var. *infestans* and *P. infestans* var. *abietis*. The first variety is Eurasian and includes a northern and a southern group. The second variety is American-Japanese and includes two formae speciales.

Introduction

Phacidium infestans sensu lato is one of the most important needle pathogens, killing conifer needles covered by snow. It is distributed in the temperate zone, killing seedlings and plants within vast areas of northern Europe, mountains in central and southern Europe, northern Asia, Japan and North America.

P. infestans sensu lato may be divided into two varieties: *P. infestans* var. *infestans* (including the type from Finland described by P. Karsten) and *P. infestans* var. *abietis*. Dearness. *Phacidium infestans* var. *infestans* is distributed in Europe and northern Asia, *P. infestans*. var. *abietis* in Japan and North America.

Björkman (1) and many others have studied the biology of this pathogen. A detailed literature review of *Phacidium infestans* sensu lato has been published recently (20).

Phacidium Infestans Var. *Infestans*

Systematics and nomenclature.–Terrier (23) separated a southern group under the name *Phacidium pini-cembrae* (Rehm) Terrier as a species distinct from *P. infestans* P. Karsten. The name is not valid: Rehm (16) did not give a description of the fungus, while Terrier gave a detailed description, but without a Latin diagnosis. It may be debated whether the southern group should be regarded as a separate species.

Authors have discriminated between *P. infestans* sensu stricto and *P. "pini-cembrae"* on the basis of hosts, production of apothecia on the adaxial or abaxial side of the needles, size of the ascospores, and irregularities as to size and number of ascospores in the asci.

It has been maintained that *P. infestans* sensu stricto only attacks two-needled pines, and *P. "pini-cembrae"* only five-needled pines. Thus Minter and Millar (11) listed only one host for *P. infestans*, the two-needled *Pinus sylvestris* L., and Millar and Minter (10) listed for *P. "pini-cembrae"* only the closely related five-needled pines *P. cembra* L. and *P. sibirica* Du Tour. Also, Dicosmo, Nag Raj, and Kendrik (4) did not list five-needled pines as hosts for *P. infestans*, nor two-needled pines as hosts for *P. "pini-cembrae"*. But in Norway the five-needled pines, *P. sibirica* and *P. pumila* (Pall.) Regel have been infected from *P. sylvestris*. On the other hand, infection of *P. sylvestris* by *P. "pini-cembrae"* has been reported from the Alps in northern Italy by Moriondo (12). It can be concluded that the occurrence on two- or five-needled pines can not be used as a discriminating character.

As to the production of apothecia on the adaxial or abaxial side of the needles, Minter and Millar (11) wrote: "*P. infestans* apothecia arise on the abaxial surface of the needles, those of *P. pini-cembrae* on the adaxial or radial surfaces". But apothecia are formed on both sides of the needles of two-needled pines. It is correct that apothecia only are formed on the adaxial (radial) surfaces of the needles of the five-needled pines, not on the convex face as maintained by Dicosno, Nag Raj, and Kendrik (4); however, in this case the cause is probably the anatomy of the needles. The five-needled pines have stomata only on the adaxial sides, whereas the two-needled pines have stomata on both sides of the needles. It can be concluded, therefore, that the production of apothecia on the adaxial or abaxial surfaces of the needles can not be used as a discriminating character in this case.

It has been claimed that the ascospores of *P. "pini-cembrae"* are smaller than those of *P. infestans*. Millar and Minter (10) gave the spore measurements as 15-30 x 4-8 µm in eight-spored asci of *P. "pini-cembrae"*. But these measurements agree well with those given for *P. infestans* by some other authors (table 1). Therefore, spore size does not seem to be a good discriminating character.

[1]Paper presented at the I.U.F.R.O. W.P. S2.06.04 Foliage Disease Conference, 29 May-2 June, 1989, Carlisle, Pennsylvania.

[2]P.O. Box 61, N 1432 Ås-NLH, Norway.

Table 1.–Sizes of the asci and ascospores of *Phacidium infestans* var. *infestans* on *Pinus sylvestris*.

Author	Asci		Ascospores	
	Length µm	Width µm	Length µm	Width µm
Karsten (1886)	90-130	18-21	22-39	8-9
Lagerberg (1912)	98-105	13-15	16-23	6-8
Terrier (1942)	130-160	13-17	11.4-28.6	4.3-8.6
Vedernikov (1965a)	72-140	12.0-24.8	11.5-27.6	5.2-9.4
Vleugel (1911) Norwegian material	109-117	16-18	18-23	8-9
Roll-Hansen, unpubl.	66-153	14-22	15-26	4.9-9.7

It has been found that *P. "pini-cembrae"* sometimes produces asci with 2-4 (-5) ascospores which are larger than the normal ones (7, 8, 10). Gremmen (7) used the name *Phragmonaevia gigaspora*. Korf (8) used *Gremmenia gigaspora*. Petrak (13), comparing collections from *P. cembra* with collections from *P. sylvestris* , found that *П. "pini-cembrae"* was conspecific with *P. infestans*; but asci with 2-4 large spores were sometimes found on *P. cembra*, apparently produced mostly in apothecia that were not fully developed nor fully ripe.

From the discussion above regarding hosts, production of apothecia on the adaxial or abaxial side of the needles, spore size, and irregular development of ascospores, I conclude that *P. "pini-cembrae"* can be regarded as conspecific with *P. infestans*. Petrak (13), Donaubauer (5), and Reid and Pirozynski (18) drew the same conclusion.

It must be admitted, however, that there seem to be some small differences between the northern, Eurasian group of *P. infestans* and the one in the mountains of central and southern Europe. *Pinus sylvestris* seems to be a better host to the northern group than *P. sibirica*. *Pinus pumila* is a relatively poor host to this group (9). The five-needled *P. cembra* is the main host to the southern group, however, the two-needled *P. sylvestris* (12) and *P. nigra* Arn. are seldom attacked (15). It is of special interest that the two-needled *P. mugo* Turra, which is very common in the Alps, has never been found attacked in that region, but has been infected when planted in Norway. It is reasonable to think that a genetic differentiation has taken place during thousands of years of independent evolution. We might speak of two races or groups - a northern and a southern group of *P. infestans* var. *infestans*.

Phacidium Infestans var. *Infestans.* The Northern Group.

Distribution.—The northern group is distributed in the northern, temperate coniferous zone from Norway, through Sweden, Finland, and European U.S.S.R., to the eastern part of Asiatic U.S.S.R.

Hosts.—The main host of the northern group of *P. infestans* var. *infestans* is *Pinus sylvestris*. But in the U.S.S.R. it is common on *P. sibirica* and has been found on *P. pumila* (9). It is also common on planted *P. contorta* Dougl. and has been found on planted *P. mugo, P. pumila,* and *P. sibirica* in Norway. Apothecia have been found on needles of *Juniperus communis* L. in Sweden, Finland, and the U.S.S.R., and on *Abies concolor* (Gord. & Glend.) Lindl. in Norway (19). Infection without fruiting has been found in Norway on *A. grandis* (Dougl.) Lindl., *A. nordmanniana* (Steven) Spach, *A. procera* Rhed., and *Picea pungens* Engelm. (19). Infection without fruiting is also known on *Picea abies* (L.) Karst. from Sweden and the U.S.S.R. (19). Dicosmo, Nag Raj, and Kendrick (4), citing Pomerleau (14), listed *Picea glauca* (Moench) Voss. and *Pinus resinosa* Ait. as hosts. Pomerleau (14) probably was in error, as in 1942 there was still much confusion as to the identity of the snow mold fungi on conifers in North America.

Phacidium Infestans var. *Infestans.* The Southern Group.

Distribution.—The southern group is found in the mountains of Central Europe (France, Switzerland, Austria, and Italy). It has probably also been reported from southern Italy on *P. nigra* under the name *P. infestans* (15). *Phacidium infestans* var. *hikmetae*, described by Bremer et al. (2) from Turkey near Ankara, may also belong to the southern group.

Hosts.—*Pinus cembra* is the main host. *Pinus sylvestris* has been attacked in the Alps. *Pinus nigra* ssp. *laricio* (Poir.) Maire has been attacked in southern Italy by *P. infestans* var. *infestans* probably belonging to the southern group.

Phacidium Infestans var. *Abietis*

Systematics and nomenclature.—The variety *P. infestans* var. *abietis* was established by Dearness (3). Reid and Cain (17) gave it the species name *P. abietis* (Dearness) Reid & Cain. They found that *P. abietis* may be distinguished from *P. infestans* by (a) apothecia only on the lower sides of the needles, (b) a papilla of the upper stromatic layer, and (c) somewhat smaller asci and ascospores.

The formation of the apothecia only on the lower side of the needles seems to be a good systematic character for *P. abietis*. *Abies concolor* is the type host; it has stomata on both sides of the needles, but apothecia are only found on the lower side. In Norwegian material of *P. infestans* on *A. concolor*, apothecia are found on both sides of the needles (19).

The value of the papilla of the upper stromatic layer as a distinguishing character may be more uncertain. Reid and Cain wrote ". . . with usually only a small central papilla of stromatic tissue being observed erumpent through epidermis."

Reid and Cain found asci to measure 90-125 x 13.5-15 µm and ascospores 18-25 x 6-7.5 µm in *P. abietis*. Measurements of European material of *P. infestans* are given in table 1. It appears that the measurements overlap significantly.

It may be a matter of opinion, but from what is said above it seems warranted to use the variety name *P. infestans* var. *abietis*. *Phacidium infestans* var. *abietis* may be divided into two formae speciales: *P. infestans* var. *abietis* f. sp. *abietis* with *Abies* spp. as the main hosts, and *P. infestans* var. *abietis* f. sp. *piceae* often producing apothecia on *Picea* spp.

Phacidium Infestans var. *Abietis* F. SP. *Abietis*

Distribution.—Northern temperate, coniferous forests in Canada and the U.S.A.

Hosts.—*Abies balsamea* (L.) Mill., *A. concolor, A. grandis, A. lasiocarpa* (Hook.) Nutt., and *Pseudotsuga menziesii* (Mirb.) Franco (17).

Dicosmo, Nag Raj, and Kendrik (4) found that the collection DAOM 71622 of *Phacidium* on *Pseudotsuga menziesii* was best referred to the European *P. "pini-cembrae."* They stated that it represented the first record of that species in North America and also the first report of *P. "pini-cembrae"* on a host other than a five-needled pine. But the authors did not mention that Reid and Cain (17) had examined DAOM 71622 and without reservation had identified it as *P. abietis*. Funk (6) stated that *P. "pini-cembrae"* occurs on *P. menziesii*. But Reid (pers. comm. 1986) has been informed by Funk that the report of *P. "pini-cembrae"* on *Pseudotsuga* had been based on the statement by DiCosmo et al. Thus, the occurrence of *P. "pini-cembrae"* on *Pseudotsuga* in North America has not been proven.

Minter and Millar (11) stated: "Reports of *P. infestans* on *Abies* and *Picea* from North America are now considered to concern a separate species, *P. abietis*." According to Reid and Cain (17) *P. abietis* had not been found on *Picea* in North America; neither does it seem to have been recorded since.

Dicosmo et al. (4), citing Pomerleau (14), listed *Picea glauca* and *Pinus resinosa* as hosts for *P. infestans*; but Pomerleau's identification of the fungus as a *Phacidium* sp. probably was incorrect, as discussed earlier

It can be concluded that *P. infestans* var. *abietis* (*P. abietis*) in North America is known only from the genera *Abies* and *Pseudotsuga*.

Phacidium Infestans var. *Abietis* F. SP. *Piceae*

Phacidium infestans var. *abietis* f. sp. *piceae* is distributed in Japan in temperate coniferous forests. It is found on *Abies sachalinensis* Masters, *Piceae glehnii* (Fr. Schmidt) Masters, *Picea jezoënsis* (Sieb. & Zucc.) Carr., *Pinus pumila*, and *Pinus strobus* L. (21). It is the only group within *P. infestans* sensu lato which forms apothecia on species of *Picea*. In collections sent to me by Takahashi I found the asci and ascospores to measure 75-138 x 10-16 (-20) µm and (10-) 16-25 x 5-8 µm, respectively, in agreement with the measurements given for *P. infestans* var. *infestans* (table 2). In these collections the apothecia were found only on the lower surfaces of the needles of *Abies sachalinensis* and *Picea jezoënsis* and on the adaxial surfaces of the needles of *Pinus pumila*. The Japanese fungus was identified as *P. infestans* var. *abietis* Dearness by Takahashi and Saho (22), and named *P. abietis* (Dearness) Reid & Cain by Takahashi (21).

Table 2.–*Phacidium infestans* P. Karsten: varieties, hosts, and distribution.

	var. *infestans*		var. *abietis* Dearness	
	Northern Eurasia	**Mountains in Central and South Europe**	**Japan**	**North America**
Abies balsamea				+
A. balsamea, inf. exp.	x			
A. concolor	+			+
A. grandis	x			+
A. lasiocarpa				+
A. nordmanniana	x			
A. procera	x			
A. sachalinensis			+	
A. sibirica, inf. exp.	x			
Juniperus communis	+			
Picea abies var. abies	x			
P. abies var. obovata	x			
P. engelmannii, inf. exp.	x			
P. glehnii			+	
P. jezoënsis			+	
P. pungens	x			
Pinus contorta	+			
P. mugo	+			
P. nigra		+		
P. sylvestris	+	+		
P. cembra		+		
P. pumila	+		+	
P. sibirica	+			
P. strobus			+	
Pseudotsuga menziesii				+

inf. exp.: Attacked in infection experiment
+ Apothecia formed
x Attacked plants, apothecia not formed

Summary

The coniferous snow blight fungus, *Phacidium infestans* P. Karsten, includes two varieties: the Eurasian *P. infestans* var. *infestans*, and the American-Japanese *P. infestans* var. *abietis* Dearness (*P. abietis* (Dearness) Reid & Cain).

Phacidium infestans var. *infestans* attacks and fruits on *Pinus* spp., and rarely on species of *Abies* and *Juniperus*. It is found in northern and in mountainous areas of Eurasia. Apothecia are formed on both the adaxial and the abaxial sides of needles of the two-needled pines, but only on the adaxial sides of the needles of the five-needled pines. The variety includes a northern group and a southern mountainous group.

The northern group of *P. infestans* var. *infestans* forms apothecia on *Abies concolor, Juniperus communis, Pinus contorta, P. mugo, P. pumila, P. sibirica,* and *P. sylvestris*. It has also been found damaging, but not fruiting on, *Abies grandis, A. nordmanniana, A. procera,* and *Picea abies* (including var. *obovata*). *Pinus sylvestris* is the main host.

The southern group of *Phacidium infestans* var. *infestans* (*Phacidium pini-cembrae* (Rehm) Terrier, not validly published) has been found fruiting on *Pinus cembra, P. nigra,* and *P. sylvestris*. *Pinus cembra* is the main host. There may be small morphological differences between this group and the northern one.

Phacidium infestans var. *abietis* may be divided into two formae speciales: the North American *P. infestans* var. *abietis* f. sp. *abietis* and the Japanese *P. infestans* var. *abietis* f. sp. *piceae*. The first forms apothecia on the lower sides of needles of *Abies balsamea, A. concolor, A. grandis, A. lasiocarpa,* and *Pseudotsuga menziesii*. The latter forms apothecia on needles of *A. sachalinensis, Picea glehnii, P. jezoënsis, Pinus pumila,* and *P. strobus*. Records of *"P. pini-cembrae"* on *Pseudotsuga menziesii* in North America seem to have been based on misidentification.

Table 2 summarizes the geographical distribution and hosts of the varieties.

Literature Cited

1. Björkman, E. 1948. Studier över snöskyttesvampens (*Phacidium infestans* Karst.) biologi samt metoder för snöskyttets bekämpande. Meddn St. SkogsforskInst. 37(2):1-136.
2. Bremer, H., Hikmet, I., Güngör, K., Özkan, H., Ozkan, M. 1947. Türkiyenin parazit mantarlari uzerinde incelemeler. (Beitraege zur Kenntnis der parasitischen Pilze der Türkei. I.) Instanbul, Üniversitesi fen Fakültesi Mecmuasi, Seri B, 12(2):122-172.
3. Dearness, J. 1926. New and noteworthy fungi - IV. Mycologia 18:236-255.
4. DiCosmo, F., Nag Raj, T. R., Kendrick, W. B. 1984. A revision of the Phacidiaceae and related anamorphs. Mycotaxon 21:1-234.
5. Donaubauer, E. 1963. Ökologische Untersuchungen in der sübalpinen Stufe zum Zwecke der Hochlagenaufforstung. Teil II:6. Über die Schneeschütte-Krankheit (*Phacidium infestans* Karst.) der Zirbe (*Pinus cembra* L.) und einige Begleitpilze. Mittl. forst. Bund.-Vers.-Anst. Mariabrunn 60:575-600.
6. Funk, A. 1985. Foliar fungi of western trees. Canadian Forestry Service, Pacific Forest Research Centre, Victoria, B.C., 159 p.
7. Gremmen, J. 1953. Some noteworthy discomycetous fungi on coniferous hosts. Sydowia 7:141-145.
8. Korf, R. P. 1962. A synopsis of the Hemiphacidiaceae, a family of the Helotiales (Discomycetes) causing needle-blights of conifers. Mycologia 54:12-33.
9. Kossinskaja, I. S. 1974. Facidioz sosny. Novosibirsk, 91 p.
10. Millar, C. S., Minter, D. W. 1980. *Phacidium pini-cembrae* . CMI Descriptions of Pathogenic Fungi and Bacteria No. 653, 2 p.
11. Minter, D. W., Millar, C. S. 1980. *Phacidium infestans*. CMI Descriptions of Pathogenic Fungi and Bacteria No. 652, 2 p.
12. Moriondo, F. 1963. Nuovi reperti fitopatologici nei boschi italiani. Annali dell' Accademia Italiana de Scienze Forestali 12:313-345.
13. Petrak, F. 1955. Über *Phacidium infestans* Karst., einen gefährlichen Parasiten der Zirbelkiefer und einige andere in seiner Gesellschaft Wachsende Pilze. Sydowia 9:518-526.
14. Pomerleau, R. 1942. Liste annotée des maladies parasitaires des arbres observées dans le Québec. Minist. Terres For. (Quebéc), Serv. For., 39 p. (Mimeographed).
15. Ragazzi, A., Moriondo, F. 1977. Un nuovo parassita del pino laricio. *Phacidium infestans* Karst. L'Italia Forestale e Montana 32:125-137.
16. Rehm, H. 1912. Zur Kenntnis der Discomyceten Deutschlands, Deutsch- Österreichs und der Schweiz. Ber. Bayer. Bot. Ges. in München 13:102-206.
17. Reid, J., Cain, R. F. 1962. Studies on the organisms associated with "snow-blight" of conifers in North America. II. Some species of the genera *Phacidium, Lophophacidium, Sarcotrichila*, and *Hemiphacidium*. Mycologia 54:481-497.
18. Reid, J., Pirozynski, K. A. 1968. Critical notes on genera of the Hemiphacidiaceae I. *Gremmenia*. Mycologia 60:526-531.
19. Roll-Hansen, F. 1987. *Phacidium infestans* and *Ph. abietis*. Hosts, especially *Abies* species in Norwegian nurseries. Eur. J. For. Path. 17:311-315.
20. Roll-Hansen, F.. 1989. *Phacidium infestans*. A literature review. Eur. J. For. Path. 19:237-250.
21. Takahashi, I. 1979. Studies on mycoflora and diseases of coniferous trees at the central part of Hokkaido, Japan. Special reference to Ascomycetes, Fungi imperfecti and Uredinales. Bull. Tokyo Univ. Forests 69:1-143.

22. Takahashi, I., Saho, H. 1969. A snow mould fungus, *Phacidium* sp. and its damage to *Abies* spp. in Hokkaido district. Abstract papers of Annual Meeting of Hokkaido Branch, Japanese Forestry Society 18:159-163.

23. Terrier, C. A. 1942. Essai sur la systématique des Phacidiaceae (Fr.) sensu Nannfeldt (1932). Thèse présentée a l'Ecole polytechnique fédérale de Zurich en vue d'obtenir le grade de docteur ès sciences naturelles. Berne, 99 p.

Life Cycle and Epidemiology of *Elytroderma Deformans* on Pines in California[1]

Robert F. Scharpf[2]

Abstract.–Elytroderma disease, caused by *Elytroderma deformans*, is a serious and widespread disease of twigs and needles of pines in western North America. The fungus persists at endemic levels as systemic infections in branches. Epidemics occur when periods of rain coincide with the presence of young foliage and mature fungus fruiting bodies. In California, these outbreaks appear only about once a decade, but when they do occur, heavy mortality and growth loss result. Although the biology of the fungus is still not fully understood, enough information exists to propose a reasonably accurate model of its infection cycle.

The needlecast fungi belonging to the family Rhytismataceae constitute a widespread group of organisms that occurs wherever their conifer hosts are found (4). Several species are important tree pathogens. In western North America, heavy losses attributed to needlecasts have been reported for timber producing stands (1, 2, 17, 18), recreational forest areas (15), conifers grown for Christmas trees (11, 13, 14), and nurseries (11). More than 25 years ago, Waters (19) wrote: "It is almost alarming that of about 50 species of needle cast fungi discussed by Darker (4), not a single life history is understood completely." That statement is still true today.

Elytroderma deformans (Weir) Darker, the cause of Elytroderma disease of pines and hereafter referred to as "ED", is one species that causes severe damage to pines throughout much of western North America, particularly to ponderosa pines (*Pinus ponderosa* Dougl. ex Laws) and Jeffrey pine (*P. jeffrey*i Grev. & Balf.)(2, 3, 15). According to Waters (19), "The life history of *E. deformans* has been somewhat of an enigma and a prime target for assumption and scientific guesses because of its seeming departure from the orthodox habits of life of the other so-called needle casts."

Unlike nearly all other members of the Rhytismataceae, ED infects branches as well as needles. From infected needles it can grow into the phloem and xylem of a branch. Once the branch and buds are invaded by ED, the new needles that are produced each year also are infected. In addition, these persistent infections stimulate an abnormal pattern of growth in branches. Apical dominance is lost and numerous lateral shoots develop on infected branches, resulting in "brooms." The presence of numerous persistent infections and the development of brooms over time results in the loss of tree vigor and increased mortality (2, 15).

Trees of all sizes and age classes are susceptible to infection and damage from ED (2, 15, 16). No strong evidence exists for resistance among native hosts, although lodgepole pine (*P. contorta* sub sp. *murrayana* (Greve & Balf.) Engelm.) in California appears highly resistant, whereas in Canada *P. contorta* sub sp. *latifolia* Engelm. is a susceptible host.

The only two other members of the genus occur in southern Europe. *Elytroderma torres juanii* Diamandis & Minter infects a few species of native pines, but differs most noticeably from ED in that only needles are infected, and that 2 years rather than 1 year are required for needle browning and fruiting body development (5). *Elytroderma lusitanicum* Fonseca -Neves is a newly described species in Portugal that infects *P. pinea* L. Like ED, it is another member of the needlecasts infecting both needles and shoots (7).

Besides being poorly understood, the life cycle and epidemiology of ED is to some degree controversial. None-the-less, most investigators familiar with the fungus generally agree that the fungus 1) produces both imperfect (pycnidial) and perfect (hysterothecial) spore stages, 2) occurs almost always on cooler, higher elevation sites on which the hosts grow, 3) occurs at low, endemic levels in the forest and only infrequently becomes epidemic, and 4) can develop as a systemic infection by growing from infected needles into branches.

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Less well understood are the following questions:

1) What is the function of the imperfect stage?
2) When do hysterothecia and ascospores mature, and what are the conditions that favor spore release, infection, and symptom expression?
3) What are the environmental or microclimatic conditions that allow for epidemic outbreaks of the disease?
4) What are the conditions besides climate that allow epidemics to develop from endemic levels of infection?
5) Is there resistance to the disease within and between species of pine?

Life Cycle

Conflicting reports exist on the function of the imperfect stage of the fungus, development of the hysterothecia, and maturation and release of ascospores. Weir (20) refers to the imperfect stage as "spermagonia," implying that the conidia function in sexual reproduction of the fungus. Waters (19) suggested that the conidia were essential for sexual reproduction rather than as infecting spores. Laurent (8) never found mature fruiting bodies of both spore stages on the same needle and, when both stages were present, only one or the other was mature. Lightle (9) found mature pycnidia with conidia in May and June. The spores appeared in tendrals or spherical droplets, and germinated in water after 3 days. Lightle (9) did not know if conidia played a role in infection.

In California, I observed what appeared to be "receptive hyphae" originating from the hymenial layer and protruding through the surface of developing hysterothecia during June when the imperfect stage was mature and releasing conidia (fig. 1). Whether these hyphae are fertilized by conidia and function as trichogynes could not be determined and needs further investigation.

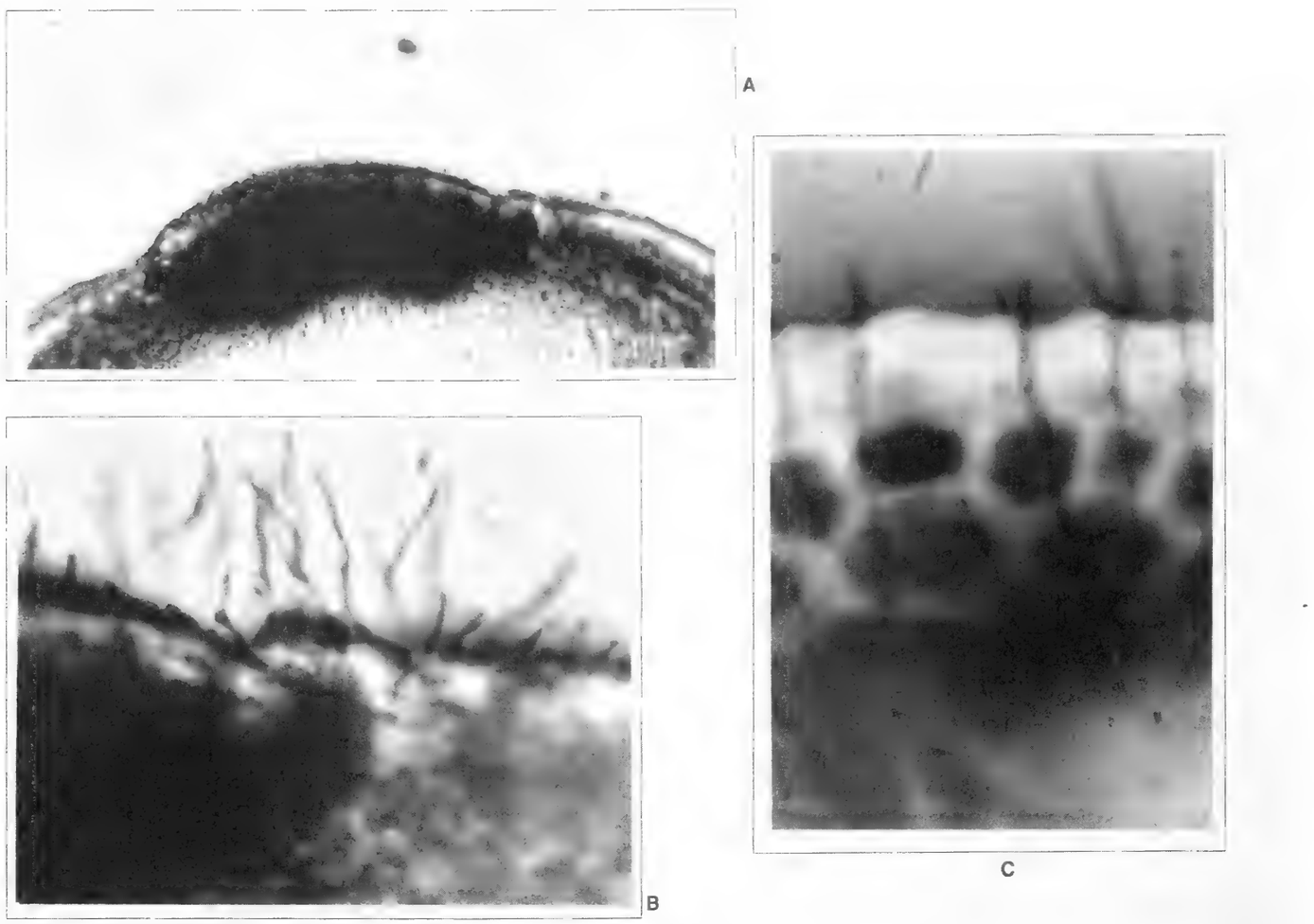

Figure 1.–A developing hysterothecium of *Elytroderma deformans* with "receptive hyphae" originating from the hymenium and protruding through the surface of the hysterothecium. (a) 100X (b) 430X (c) 900X.

Weir (20) reported that hysterothecia on needles of ponderosa pine infected the previous year in the Pacific Northwest bore fully mature asci with well developed spores in early spring. However, he reported that hysterothecia and mature spores could be found at any season of the year, and that the fruiting bodies resist drying and release spores only during periods of abundant moisture. Greatest numbers of spores were released during the May-June rainy season, however, when early vegetative growth of the host was present. Weir (20) also found that temperature regulated spore release. After 4 hours at 5 C no spores were released, whereas at 27 C abundant spores were discharged. Weir (20) concluded that warm summer rains stimulated spore release.

Waters (19) found mature ascospores in hysterothecia from July to April in Montana. Maximum spore discharge occurred from fall to early April. On the other hand, Waters (19) found that "greenhouse" conditions prevented development of hysterothecia in infected needles and he concluded that hot, dry weather in spring and fall might be important in preventing infection. Lightle (9) found mature hysterothecia and ascospores on needles only from September through December. He trapped spores mostly during rains in September and October, some in November and December and a few in January, March, April and May. Laurent (8) suggested that hot, dry weather or other variations in local and regional weather are responsible for time of hysterothecial development and maturity.

My investigations, although somewhat limited, showed maturation of hysterothecia to be much earlier in several locations in California than Lightle (9) reported (figs. 2 and 3). However, no mature hysterothecia were found in early spring as reported by Weir (20). July 28 was the earliest spore release I noted for most hysterothecia moistened and placed in a growth chamber at about 10 C. Although systematic spore release and germination

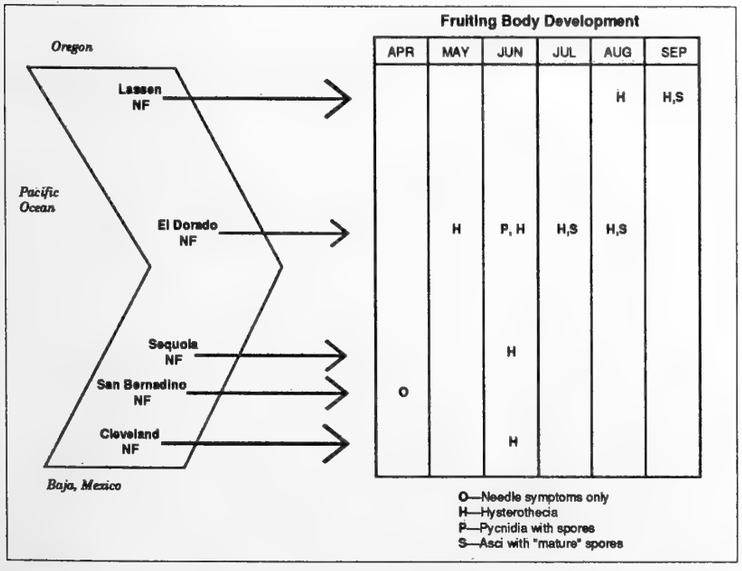

Figure 2.–Development of fruiting bodies of *Elytroderma deformans* in California in 1988.

tests were not conducted, many hysterothecia collected on needles in August and September contained asci with mature spores.

Germination

Information on conditions influencing germination of ascospores of ED is scant and somewhat contradictory. Weir (20) reported that ascospores were highly resistant to drying and remained viable in dried herbarium material for at least a year. He also found that moist spores germinated at 35 C after 4 days. On the other hand, Lightle (9) reported almost no germination at 35 C, whereas at 15-21 C nearly all ascospores germinated in a 2% sucrose solution after 24 hours. Laurent (8) was unable to germinate ascospores.

Infection

Essentially nothing is known about the processes and conditions involved in the infection of conifer hosts by ED. Some observations and studies have been made on infection in the field, however. According to Weir (20), new infections could occur anytime from the appearance of new needles until the end of the growing season. In a field test with 3-year old seedlings of ponderosa pine inoculated in May, he found that needle symptoms first appeared in September and mature hysterothecia and ascospores developed the following May-June. Older needles were uninfected. Lightle (9) suggested that germination and infection were unlikely in winter because of cold temperatures, and were improbable in spring because of limited spore discharge. Gordon and Laurent (2) reported unsuccessful inoculations of needles more than 3 weeks old. They suggested that infection waves may occur when asocarp development is retarded by unfavorable weather so that infected needles remain on the tree and produce ascospores in the spring. How ED penetrates and infects needles is not known, but most other members of the needlecasts form appressoria and penetrate needles directly (6, 10, 21).

Roth (12) reported that infections on trees in the understory occur at infrequent and unpredictable intervals, and that young seedlings do not become infected. However, Scharpf and Bega (16) found that of surviving 2-year old Jeffrey pines planted near infected overstory in California, about two thirds of the trees became infected after 14 years.

Growth in Culture

Attempts to grow ED in culture have met with mixed results. Weir (20) claimed he could grow the fungus from germinated spores on a pine needle extract medium,

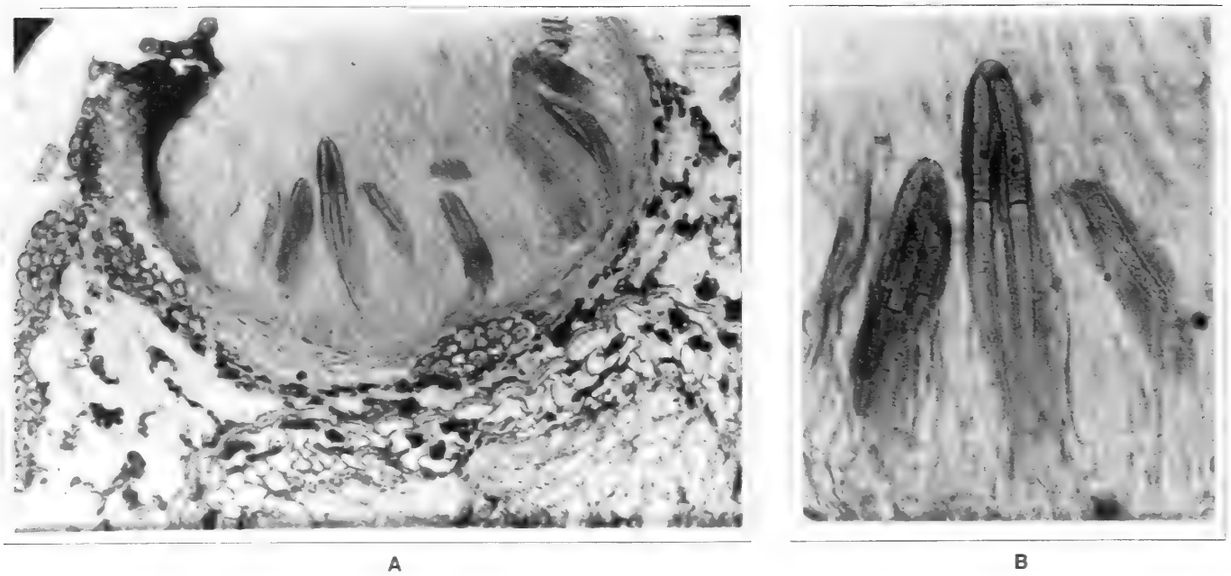

Figure 3.–(a) Cross section of a mature hysterothecium of *Elytroderma deformans* on Jeffery pine; several asci contain well-developed ascospores, July, 1988, (200X). (b) Enlarged view of a well developed ascus (center) containing elongate, 2-celled ascospores (500X).

but after about 8 months the mycelium died. Laurent (8) also reported successful culturing of the fungus from infected needles on media made from pine needle extract and pine needles in agar. Pycnidia apparently developed in some of Laurent's cultures. On the other hand, Lightle (9) failed in his efforts to culture the fungus from different portions of the host, on different media, and at different temperatures. Similarly, several attempts I made to culture ED from infected needles failed when several synthetic media were used at different temperatures. White tufts of mycelium of ED grew from the ends of cut segments of needles but failed to grow on the media used (fig. 4). In Europe, Fonseca-Neves (7) was able to grow *E. lusitanicum* and produce pycnidia on media made from potato-dextrose-agar supplemented with macerated pine needles.

Figure 4.–The mycelium of *Elytroderma deformans* grew from the cut ends of an infected Jeffrey pine needle at 15C, but did not grow on potato-dextrose-agar medium.

Infection Cycle

Even though numerous conflicting and incomplete reports appear in the literature, I believe enough information exists to propose a reasonably accurate model of the infection cycle of ED, including some of the conditions that regulate the infection process in California and possibly elsewhere. Numerous field observations and reports indicate that endemic levels of the disease are the norm and only occasionally do epidemic outbreaks occur.

What then are the conditions that are required for epidemics to take place? I suggest that several events need to occur at the same time before an epidemic is possible (fig. 5). In particular, mature hysterothecia with viable spores must be present when spring rains occur and when young (less than 1 month old) foliage is present. In areas where ED is endemic on pines in California, it is quite rare for these three conditions to occur at the same time . Susceptible young pine needles usually are not present until early June to early July in the areas where the disease occurs. By then the rainy season has ended and conditions are unfavorable for spore dispersal and infection. During earlier rains, neither susceptible foliage nor spores are present for infection to take place. Similarly, later in the season, even if summer thunderstorms occur and spores are dispersed, the new foliage is mature and no longer susceptible. In fall and winter, even though some fruiting bodies may contain spores, conditions are too cold for spore release, germination, and infection. Therefore, only about a month is usually available for pines to become infected by ED, and the coinciding of favorable weather, spore release and susceptible foliage that results in an epidemic outbreak is a rare event. The conditions that regulate infection of pines by this fungus

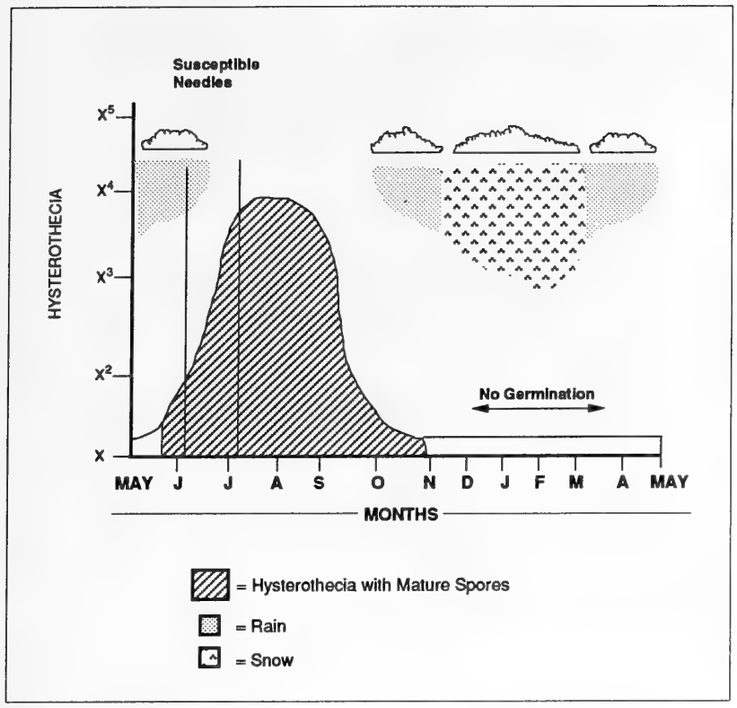

Figure 5.–Proposed infection cycle of *Elytroderma deformans* in California. Infection takes place only when rain occurs during the period when mature hysterothecia are present and needles are susceptible.

are apparently the same that regulate infection by *Lirula abietis-concoloris* (Mayr ex Dearn.) Darker, a similar needlecast fungus on *Abies* sp. in California (14).

None-the-less, some infection occurs on some pines nearly every year (16). Sufficient levels of inoculum appear to be present from these annual infections, plus that occurring from systemic infections and brooms, to cause epidemics when conditions favorable for infection are present.

Conclusions

Even though ED is recognized as a serious pathogen of commercial conifers in western North America, questions remain about its biology, epidemiology, and control. Our knowledge about this fungus and the disease it causes is hardly more than that known by Weir when he discovered and studied it in the early 1900's. But we do have enough information to propose a reasonably accurate model of the infection cycle. As the 21st century approaches, we will need to do a better job of reducing losses from forest diseases, insects, and other pests if we are to provide an adequate supply of timber and a healthy forest environment for future generations. To achieve these goals more research is needed on this and other forest pest problems to develop the knowledge and methodology on how best to prevent or mitigate losses to our invaluable forest resources.

Literature Cited

1. Brandt, R. W. 1960. The Rhabdocline needle disease of Douglas-fir. SUNY College Forestry at Syracuse. Tech. Publ. 84. 66 p.

2. Childs, T. W. 1968. Elytroderma disease of ponderosa pine in the Pacific Northwest. USDA Forest Serv. Res. Paper PNW-69. 45 p.

3. Childs, T. W., Shea, K. R., Stewart, J. L. 1971. Elytroderma disease of ponderosa pine. USDA Forest Serv., For. Pest Leaflet 42. 6 p.

4. Darker, G. D. 1932. The Hypodermataceae of Conifers. Contrib. Arnold Arboretum 1:1-131.

5. Diamandis, S. 1981. *Elytroderma Torres-juanii* Diamandis & Minter. A serious attack on *Pinus brutia* in Greece. p. 9-12 In: C.S. Millar (ed). Current Research on Conifer Needle diseases. Proc. IUFRO Working Party on Needle Diseases, Sarajevo, 1980. Forestry Dept., Univ. Aberdeen, Aberdeen, Scotland.

6. Diwani, S. A., Millar, C. S. 1986. Infection processes of three *Lophodermium* species on *Pinus sylvestris* L. p. 22-27 In: G. W. Peterson (ed.). Recent research on conifer needle diseases. USDA Forest Serv. Gen. Tech. Rep. WO-50. 106 p.

7. Fonseca-Neves, N. 1987. *Elytroderma lusitanicum* sp. nov. causing needle and shoot disease on *Pinus pinea* in Portugal. For. Abstr. 48:239-240.

8. Laurent, T. H. 1962. Studies of the developmental morphology and life history of *Elytroderma deformans*. M.S. thesis, Montana State University, Bozeman. 22 p.

9. Lightle, P. C. 1954. The pathology of *Elytroderma deformans* on ponderosa pine. Phytopathology 44:557-569.

10. Millar, C. S. 1986. *Lophodermella* species on pines. p. 45-55 In: G. W. Peterson (ed.). Recent research on conifer needle diseases. USDA Forest Serv. Gen. Tech. Rept. WO-50. 106 p.

11. Nicholls, T. H., Skilling, D. D. 1974. Control of *Lophodermium* needlecast disease in nurseries and Christmas tree plantations. USDA Forest Serv. Res. Paper NC-110. 11 p.

12. Roth, L. F. 1959. Perennial infection of ponderosa pine by *Elytroderma deformans*. Forest Sci. 5:182-191.

13. Scharpf, R. F. 1986. Effect of a foliage disease caused by *Lirula abietis-concoloris* on growth of white fir in California. Plant Dis. 70:13-14.

14. Scharpf, R. F. 1988. Epidemiology of *Lirula abietis-concoloris* on white fir in California. Plant Dis. 72:855-858.

15. Scharpf, R.F., Bega, R. V. 1981. Elytroderma disease reduces growth and vigor, increases mortality of Jeffrey pines at Lake Tahoe Basin, California. USDA Forest Serv. Res. Paper PSW-115.

16. Scharpf, R. F., Bega, R. V. 1988. Elytroderma disease in young, planted Jeffrey pine, South Lake Tahoe, California. USDA Forest Serv. Res. Note PSW-399. 2 p.

17. Shaw, C. G., Leaphart, C. D. 1960. Two serious foliage diseases of western white pine in the inland empire. Plant Dis. Rep. 44:655-659.

18. Stephan, B. R., Osorio, M. 1990. Appressoria and their possible use for identification of the Rhytismataceae (Ascomycetes). p. 108-111. In: W. Merrill and M. Ostry (eds.). Recent Research on Foliage Diseases. USDA For. Serv. Gen. Tech. Rep. WO-56, 145 p.

19. Wagener, W. W., Childs, T. W., Kimmey, J. W. 1949. Notes on some foliage diseases of forest trees on the Pacific slope. Plant Dis. Rep. 33:195-197.

20. Waters, C. W. 1962. Significance of life histories of *Elytroderma deformans*. Forest Sci. 8:250-255.

21. Weir, J. R. 1916. *Hypoderma deformans*, an undescribed needle fungus of western yellow pine. J. Agri. Res. 4:277-288.

Interrelationships Among *Lophodermium Seditiosum*, *L. Pinastri* and *Cyclaneusma Minus* in Pine Plantations (*Pinus Sylvestris* L.) in Poland[1]

Tadeusz Kowalski[2]

Abstract.–The study was conducted in sixteen 3- to 5-year-old and sixteen 6- to 10-year-old plantations in southern Poland. In each plantation the frequency of occurrence of *Cyclaneusma minus, Lophodermium seditiosum*, and *L. pinastri* on needles in the litter was determined. On the basis of these data the statistical correlation in coexistence of these fungi in the plantations was calculated. In 3- to 5-year-old plantations the correlations in coexistence of *C. minus* and *L. pinastri, C. minus* and *L. seditiosum*, and *L. seditiosum* and *L. pinastri* were significant ($\partial = 0.05$), the first one being directly and the remaining two inversely proportional. In 6- to 10-year-old plantations only the correlation in coexistence of *L. seditiosum* and *L. pinastri* was statistically significant.; it was inversely proportional.

Introduction

Cyclaneusma minus (Butin) DiCosmo, Peredo & Minter, *Lophodermium seditiosum* Minter, Staley & Millar, and *L. pinastri* (Schrad.) Chev. are among the fungal species most frequently isolated from unhealthy needles of *Pinus sylvestris* L. in Poland (2,3). They also are the fungi most frequently fructifying on needles in the litter (4,5). The frequency of their occurrence, however, depends considerably on the age of stand, e.g. *C. minus* and *L. pinastri* only occur sporadically in forest nurseries, *L. seditiosum* occurs sporadically in stands over 30 years of age, and the occurrence of *C. minus* distinctly decreases as stands age. All three species, however, may occur abundantly in young pine plantations (4). Such plantations were the object of this study. Its purpose was to determine the correlation of coexistence of these three species of fungi. In general, there is lack of such information. Usually coexistence of various species of fungi has been studied in relation to a single needle (7,10,11,12).

Materials and Methods

The needles for this study were collected in sixteen 3- to 5-year-old and sixteen 6- to 10-year-old plantations situated in the following four forest management units in southern Poland: Brynek, Chrzanów, Lezajsk, and Swierklaniec. The plantations were composed of *P. sylvestris* either exclusively, or with a mixture of other species up to 20%, and grew on fresh coniferous forest sites. From the end of August to November 1984, needle collections were made in 10 places in each plantation, 50 needles in each place. The needles were collected from layer AoF, i.e., they had been lying in the litter for some time. Freshly fallen needles were not taken into account. The collected needles were then analyzed mycologically in the laboratory. Cultures of *C. minus, L. seditiosum*, and *L. pinastri* were isolated from these needles also and reared together in Petri dishes on malt agar medium at 21 C.

Moreover, the following were done: 1) compilation of coefficient of correlation between frequency of occurrence in plantations of *C. minus* and *L. pinastri, C. minus* and *L. seditiosum*, and *L. pinastri* and *L. seditiosum*, 2) testing whether this correlation was significant at level of confidence $\partial = 0.05$ (14), and 3) in case of a significant correlation, regression was computed (fig. 3).

Results

Plantations 3 to 5 years old.—Numbers of needles of *P. sylvestris* colonized by *C. minus, L. seditiosum*, and *L. pinastri* in individual plantations are shown in table 1. Out of the 3- to 5-year-old plantations tested, in ten cases *C. minus* was the most numerous species; on the average it was present on 327 needles (out of 500 tested). In six plantations *L. seditiosum* was the most numerous species occurring on 260 needles on the average. The data in table 1 show that the frequent occurrence of *C. minus* coincided with the relatively frequent occurrence of *L. pinastri*, and the scarce occurrence of *L. seditiosum*. On the other hand, in plantations where *L. seditiosum*

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dominated, *C. minus* and *L. pinastri* were less numerous. This correlation corresponds with data in table 2, which shows that both species of *Lophodermium* occurred on a single needle only occasionally. Frequently individual needles were colonized by *C. minus* and *L. pinastri*. However, their distribution on needles varied. On some needles *C. minus* occupied sections up to 40 mm long, leaving the remaining part to *L. pinastri*. On other needles *C. minus* occupied several short sections about 3-6 mm long (totaling 50%, and sometimes to 75% of the needle length) while *L. pinastri* fructified on the remaining sections (fig. 1). Cultures reared on malt agar showed that during the initial growth period *L. pinastri* distinctly hindered the development of cultures of *C. minus* and *L. seditiosum*. After 3 to 4 weeks only an inhibition zone 4 -9 mm wide formed between them (fig. 3).

The correlation coefficients were computed on the basis of the numbers of needles colonized in plantations by individual fungus species. The coefficient of correlation between occurrence of *C. minus* and *L. pinastri* was positive, while those for *C. minus* and *L. seditiosum*, and *L. seditiosum* and *L. pinastri* were negative (table 3). Subsequent analysis showed that correlations in coexistence of these fungi in 3- to 5-year-old plantations were statistically significant ($\partial = 0.05$). The correlations between mutual occurrence of these species are shown as regression lines in Fig. 3. They, as well as correlation coefficients (table 3), showed that correlation in coexistence of *C. minus* and *L. pinastri* is directly proportional (fig. 3B) while the remaining two correlations were inversely proportional (fig. 3A, C).

Plantations 6 to 10 years old.—In the 6- to 10-year-old plantations studied, *C. minus* was the dominant species on needles in the litter in three plantations, *L. seditiosum* in two, and *L. pinastri* in eleven (table 1). In general, in those plantations where *C. minus* was numerous *L. pinastri* also was numerous, while in places where *L. seditiosum* was numerous, the other two species were less numerous. Analysis showed, however, a significant correlation ($\partial < 0.05$) only in the coexistence of *L. seditiosum* and *L. pinastri*. This correlation (regression line on fig. 3D) was inversely proportional. The correlation in coexistence of *C. minus* and *L. pinastri*, and *C. minus* and *L. seditiosum* in 6- to 10-year-old plantations was insignificant ($\partial > 0.05$).

Discussion

The fungus species studied play a different role in disease process of needles of *P. sylvestris*. *Lophodermium seditiosum* is the most serious cause of pine needlecast. *Cyclaneusma minus* may occur on needles as an endophyte not causing disease (13), or causes "autumn" needlecast, especially of 2-year-old needles (1,5). *Lophodermium pinastri* colonizes mainly older needles and needles previously infected by other pathogens, and therefore plays a secondary role in the disease process (2,6,9). These three species occurred on needles in the litter in all the plantations studied in southern Poland (table 1). Differentiation in their frequency in plantations of different management units, as well as within a single management unit, confirms, among other things, a considerable influence of local conditions on disease process in needles of *Pinus sylvestris* (15). In spite of different frequency of occurrence of each of the fungus species studied, the analysis showed that there are close correlations in their coexistence, especially in 3- to 5-year-old plantations. These correlations, directly proportional (between *C. minus* and *L. pinastri*), or inversely proportional (between *C. minus* and *L. seditiosum*, and *L. seditiosum* and *L. pinastri*), are statistically significant ($\partial = 0.05$). It seems that in individual cases these correlations may originate from different reasons. *Lophodermium seditiosum* is able to colonize needles and cause their death earlier than *C. minus* (12), and therefore, when *L. seditiosum* colonizes a large percentage of the needles resulting in maximum needlecast in the spring, there is less infection of needles by *C. minus* and less autumn needlecast connected with this fungus. This seems to be a main reason for the inversely proportional correlation between occurrence of *C. minus* and *L. seditiosum* in pine plantations. In such plantations there also is only a slight possibility for colonization of needles by *L. pinastri*, which colonizes older needles. Moreover, as the study showed (table 2), the needles colonized by *L. seditiosum* are rarely colonized secondarily in nature by *L. pinastri*. Therefore, the correlation in coexistence of these two species in plantations is also inversely proportional (fig. 3C, D). On the other hand, *L. pinastri* is highly competitive in needles initially infected by *C. minus* as shown by isolation of fungi from such needles (3), as well as by the high frequency of their fructification on dead needles (fig. 1, table 2). Similar behavior of *L. pinastri* has been observed on needles initially colonized by other pathogens, e.g., *Dothistroma pini* Hulbary, *Lophodermella sulcigena* (Rostr.) Höhn. (8,10). It cannot by excluded that in these cases *L. pinastri* is aided in competition for substratum by producing antibiotic substances which hinder the growth of other fungi, as was observed during an the initial period of its growth on malt agar (fig. 2). The reason for a directly proportional correlation in coexistence of *C. minus* and *L. pinastri* in pine plantations (fig. 3B) may be due to the frequent coexistence of these two species on a single needle. *Cyclaneusma minus* and *L. pinastri* also frequently coexisted on the same needles in 6- to 10-year-old plantations (table 2). The correlation in their coexistence in such plantations, as in case of *C. minus* and *L. seditiosum*, was statistically insignificant. This may have been influenced, among other things, by the closing of the canopy in some plantations resulting in the early death of needles because of lack of light. Further studies, conducted in other areas, should show whether the correlations in coexistence of fungi reported here are typical only for the area tested in 1984, or whether they may be generalized.

Literature Cited

1. Kistler, B. R., Merrill, W. 1978. Etiology, symptomatology, epidemiology and control of Naemacyclus needlecast of Scotch pine. Phytopathology 68:267-271.

2. Kowalski, T. 1982. Fungi infecting *Pinus silvestris* needles of various ages. Eur. J. For. Path. 12:182-190.

3. Kowalski, T. 1987. Mikoflora chorych i zamierajacych igiel *Pinus sylvestris* L. w drzewostanach Polski poludniowej. Zesz. Nauk. AR w Krakowie nr 212 Lesnictwo z. 12:51-62.

4. Kowalski, T. 1988. Zur Pilzflora toter Kiefernnadeln. Z. Mykol. 54:159-173.

5. Kowalski, T. 1988. *Cyclaneusma* (*Naemacyclus*) *minus* an *Pinus sylvestris* in Polen. Eur. J. For. Path. 18:176-183.

6. Kowalski, T., and Lang, K. J. 1983. Über die Mykoflora in den Nadeln unterschiedlich alter Kiefern (*Pinus sylvestris* L.). Phytopath. Z. 107:9-21.

7. Lazarev, V. 1986. Ecology and succession of some fungi causing pine needle diseases in Yugoslavia. p. 41-44 In: G. W. Peterson (ed.). Recent Research on Conifer Needle Diseases. USDA For. Serv. Gen. Tech. Rep. WO-50. 106 p.

8. Millar, C. S. 1970. Role of *Lophodermella* species in premature death of pine needles in Scotland. Dept. For., Aberdeen Univ., Aberdeen, Scotland, Rep. For. Res. p. 175-178.

9. Millar, C. S., Richards, G. H. 1975. The incidence of *Lophodermium* types in attached pine needles. Mitt. BundForsch-Anst. Forst- u. Holzw. 108:57-76.

10. Murray, J. S. 1967. *Dothistroma pini* Hulbary - Its occurrence in Europe. XIV IUFRO - Kongress. Munchen.

11. Osorio, M., Rack, K. 1980. Beobachtungen über Wechselwirkungen dreier Nadelpilze der Kiefer in vitro. Eur. J. For. Path. 10:242-252.

12. Rack, K. 1981. Interaction between *Naemacyclus minor* and *Lophodermium pinastri* during "pine needle cast." p. 103-111 In C. S. Millar (ed.). Current Research on Conifer Needle Diseases. Dept. For., Aberdeen Univ., Aberdeen, Scotland. 113 p.

13. Rack, K., Scheidemann, U. 1987. Über Sukzession und pathogene Eigenschaften Kiefernnadeln bewohnender Pilze. Eur. J. For. Path. 17:102-109.

14. Rumszyski, L. Z. 1973. Matematyczne opracowanie wynikow eksperymentu. Warszawa, Wyd. Nauk.-Techn. 197 p.

15. Schütt, P. 1964. Der Schüttebefall der Kiefer in Abhängigkeit von Herkunft und Anbauort. Forstw. Cbl. 83:140-163.

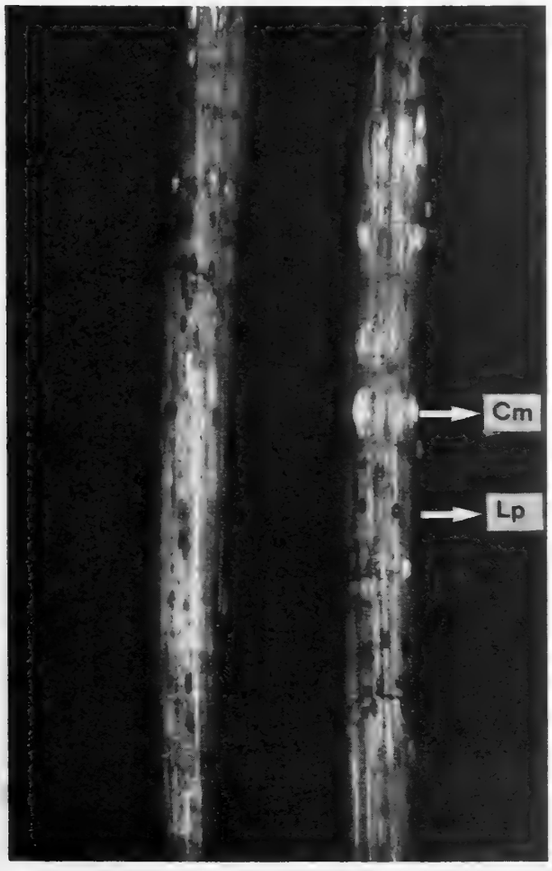

Figure 1.–Needles mutually colonized by *Cyclaneusma minus* (Cm) and *Lophodermium pinastri* (Lp) with distinct fructifications

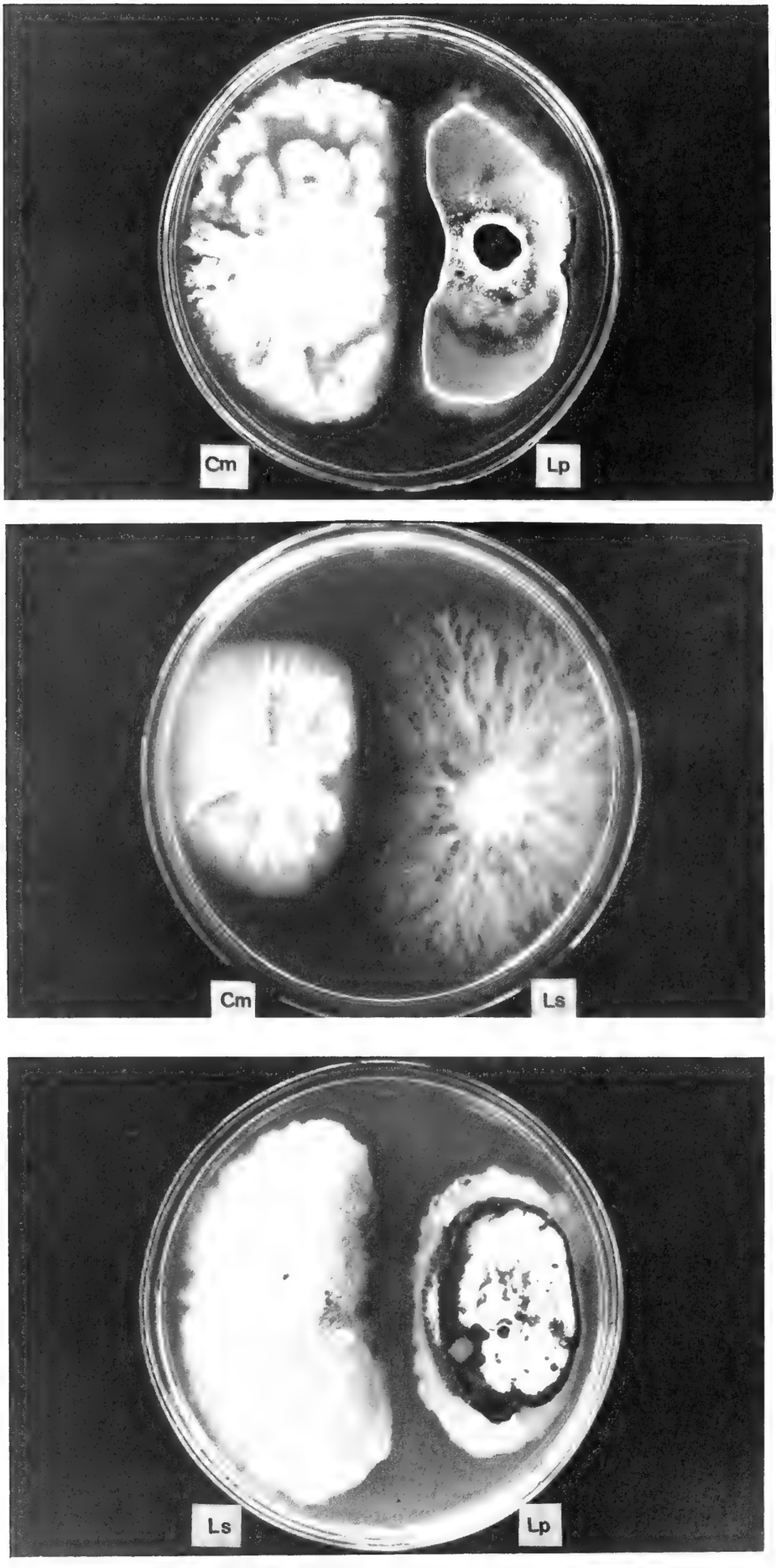

Figure 2.–Three-week-old cultures on malt agar medium (@21 C), Cm = *Cyclaneusma minus*, Lp = *Lophodermium pinastri*, Ls = *L. seditiosum*

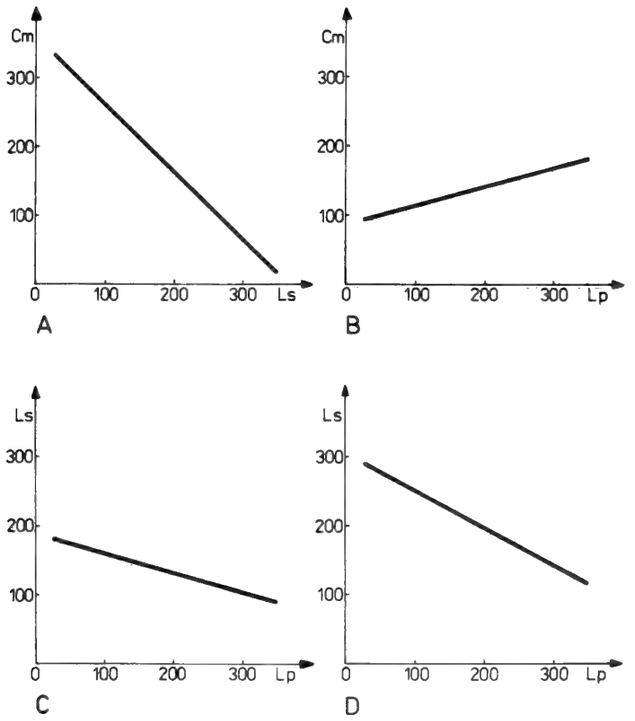

Figure 3.–Regression lines showing correlation in coexistence of *Cyclaneusma minus* (Cm), *Lophodermium pinastri* (Lp), and *L. seditiosum* (Ls) on needles in the litter of 3- to 5-year-old plantations (ABC), and 6- to 10-year-old plantations (D).

Table 1.–Number of needles in the litter with fructifications of *Cyclaneusma minus, Lophodermium seditiosum* and *L. pinastri* (500 needles tested in each plantation)

Plantation	Management	Age of Plantations					
		3- to 5-year old			6- to 10-year old		
No.	Unit	*C. minus*	*L. seditiosum*	*L. pinastri*	*C. minus*	*L. seditiosum*	*L. pinastri*
1	Brynek	202	118	148	127	2	383
2		82	316	64	348	21	401
3		113	122	102	336	101	341
4		154	244	121	317	8	393
5	Chrzanow	328	17	220	212	21	213
6		308	16	209	279	2	312
7		309	14	223	285	7	227
8		328	21	239	205	2	295
9	Lezajsk	79	266	111	118	199	229
10		143	292	113	165	39	330
11		129	322	147	219	286	81
12		307	43	177	30	285	163
13	Swierklaniec	365	23	197	149	4	258
14		353	16	132	319	7	245
15		380	38	123	374	33	149
16		390	17	145	188	4	214
average/500 needles tested in plantations with predominance of:							
	C. minus	327	32	181	326	16	207
	L. seditiosum	117	260	110	125	286	122
	L. pinastri	—	—	—	222	37	306

Table 2.–Percentage of needles in the litter mutually colonized by fungi

Fungi	Age of plantations							
	3- to 5-year old				6- to 10-year old			
	Management Unit							
	Brynek	Chrzanow	Lezajsk	Swierklaniec	Brynek	Chrzanow	Lezajsk	Swierklaniec
C. minus and *L. pinastri*	10.6	37.4	10.6	21.6	45.9	32.8	11.0	17.0
C. minus and *L. seditiosum*	8.4	3.0	5.5	2.5	2.8	0.4	5.9	0.3
L. pinastri and *L. seditiosum*	0.4	0.0	0.4	0.0	0.2	0.2	0.5	0.2

Table 3.–Correlation coefficients for frequency of occurrence of *Cyclaneusma minus*, *Lophodermium seditiosum*, and *L. pinastri*

Correlation coefficient(r)	Age of plantations	
	3- to 5-year old	6- to 10-year old
^{r}CmLp^{x}	0.64	0.22
^{r}CmLs	-0.90	-0.45
^{r}LsLp	-0.68	-0.57

^{x}Cm = *Cyclaneusma minus*
Lp = *Lophodermium pinastri*
Ls = *Lophodermium seditiosum*

Cyclaneusma Needlecast in Scots Pine Christmas Tree Plantations in the Lake States[1]

M.E. Ostry[2], T.H. Nicholls[2], J.C. Carlson[3], and G.C. Adams[4]

Abstract.–Cyclaneusma needlecast , which can damage Scots pine, is becoming increasingly important in the Lake States. The complex life cycle of the fungus has hindered the development of a control program. Although research has progressed, additional information is needed before effective, economical controls can be recommended for nurseries and plantations.

Introduction

About one-third of the Christmas trees produced in the United States come from the Lake States of Minnesota, Wisconsin, and Michigan. Cyclaneusma needlecast, caused by the fungus *Cyclaneusma minus* (Butin) DiCosmo, Peredo & Minter (= *Naemacyclus minor* Butin), has become a serious problem in the last 10 years on Scots pine (*Pinus sylvestris* L.), the predominant species grown for Christmas trees in the Lake States. *Cyclaneusma minus* causes premature yellowing and casting of 2- and 3-year-old needles (4). Repeatedly infected trees retain only a single year's complement of needles, which reduces tree vigor and quality. This reduction results in serious financial losses to growers.

Cyclaneusma minus was not considered an important pathogen in Christmas tree plantations in the Lake States until it was first reported in Michigan in 1977 (2). Increased reports of its damage to Christmas tree plantings throughout the Lake States and in Pennsylvania (3) prompted us to study its biology and control.

The objective of this paper is to describe the distribution and impact of Cyclaneusma needlecast in the Lake States and to summarize the findings of our cooperative research on the biology and control of *C. minus*.

Distribution

Cyclaneusma needlecast was either not frequently reported, or most likely confused with normal needle senescense, winter injury, or damage caused by *Lophodermium seditiosum* Minter, Staley, & Millar in Michigan and Minnesota until the early 1980's, when reports of damage attributed to *C. minus* increased. A 1987 survey revealed that *C. minus* was present in Scots pine plantations throughout most of Michigan (1). Although a formal survey has not been undertaken in Minnesota, Cyclaneusma needlecast was first reported there in 1980 and has since been found in plantations throughout the state.

Before 1984, Cyclaneusma needlecast was present in Wisconsin, but incidence and impact were low. In 1985, a survey of disease incidence in Scots pine Christmas tree plantations in central Wisconsin was conducted by the Wisconsin Department of Natural Resources (5). Twenty-two plantations, ranging in age from 4 to 9 years old, were surveyed for the presence of apothecia of *C. minus*. In each plantation, 0.2 percent of the trees were sampled. *Cyclaneusma minus* was detected in all the plantations and on an average of 95 percent of all trees sampled in each plantation. Isolation results showed a higher frequency of recovery of *C. minus* from the 1983 needles than the 1984 needles. A follow-up survey in 1985 revealed that *C. minus* was distributed generally throughout Wisconsin wherever Scots pine was grown.

Because of the 6- to 12-month latent period (4,6) new infections of needles by *C. minus* often go unnoticed. At the present time no nurseries that we are aware of are protecting their Scots pine stock from Cyclaneusma needlecast. The wide distribution of the disease may be the result of shipping infected planting stock. In April 1988, isolations were made from 2- and 3-year-old Scots pine seedlings from four different seed sources obtained from a Wisconsin nursery. *Cyclaneusma minus* was recovered from seedlings of two of the four sources, confirming its presence in the nursery and its potential for spread on planting stock.

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Economic Impact

Cyclaneusma minus is particularly damaging to Scots pine plantations because it can infect needles of any age throughout the tree (6). Severely affected trees may have only a single year's complement of needles at harvest, which lowers the grade of the tree or makes the tree unmarketable.

In 1985, the economic loss due to Cyclaneusma needlecast on one Christmas tree farm in central Wisconsin was determined by rating 4,500 Scots pine trees for needlecast. Based on the existing tree grading system and 1985 tree prices, Cyclaneusma needlecast reduced the potential value of the farm by $420,775 or 26%. In 1987 in Michigan, it was estimated that if loss due to Cyclaneusma needlecast was also 26%, the yearly economic loss may be as high as $13 million in harvestable trees (1).

Biology

Understanding the life cycle of *C. minus* is essential for developing an effective and economical control program. Studies have been designed to determine the timing of spore discharge and infection of needles to develop a protective fungicide program for the Lake States. Various types of spore traps have been used for the last 4 years in Wisconsin and Michigan in attempts to determine the relationships between weather, spore release, and infection.

Unlike other needlecast fungi we have studied, *C. minus* does not have a specific pattern of spore release. Spores are released with rainfall or during periods of prolonged dew and high humidity throughout the growing season. It has not been possible to predict when peak spore dispersal will occur. We found that major spore releases are possible at any time throughout the growing season. Because spore release and infection are unpredictable, it is difficult to time fungicide applications to protect trees from infection.

Chemical Control

Several fungicides have been tested at various rates and times throughout the growing season in Wisconsin and Michigan over the last several years. The variable yearly pattern of spore release and infection, winter injury to trees, and recent droughts that reduced infection have complicated this research in the Lake States. However, our results show that chlorothalonil effectively controls *C. minus* when applied five to seven times during the growing season either from the ground or from the air.

In Pennsylvania, five chlorothalonil sprays beginning in early spring before budbreak have been effective. This spray schedule is now recommended for growers in Pennsylvania (7) and is being tested in the Lake States. In the Lake States, this spray schedule will also help growers to control two other serious needlecast diseases, brown spot caused by *Scirrhia acicola* (Dearn.) Siggers and needlecast caused by *Lophodermium seditiosum* .

Current Research

The focus of current research in the Lake States is on increasing our understanding of the biology of *C. minus* under our regional environmental conditions so that we can develop an effective and economical control program for nurseries and Christmas tree plantations. Our studies revealed many similarities in the behavior of the disease in the Lake States and Pennsylvania. Our objective now is to fine tune our control recommendations in the Lake States so that a minimum number of fungicide applications is needed.

We still lack critical information about conditions necessary for spore dispersal, germination, and infection. The long latent period of the disease makes it difficult to assess the incidence of infection and complicates the evaluation of fungicide treatments.

Control of Cyclaneusma needlecast in nurseries may require a different set of control recommendations from those used in plantations because of differences in economics, cultural practices, and environmental conditions. Research will be directed at developing fungicidal controls for use in nurseries to avoid the distribution of infected planting stock.

A study is underway to determine the effects of soil fertility on the incidence and severity of Cyclaneusma needlecast in Wisconsin. Some questions exist on what role, if any, nutrition and stress play in the disease. To determine this, various levels and formulations of fertilizers are being applied to trees. Tree growth and disease incidence data are being collected.

Management practices aimed at protecting Scots pine Christmas tree plantations from infection by *C. minus* need to be fully integrated into the total management of these plantations. Growers are now managing their plantations to minimize tree injury caused by many damaging agents, and these practices must be integrated into an effective, economical plan.

The Wisconsin Department of Natural Resources, in cooperation with the University of Wisconsin and the Wisconsin Christmas Tree Producers Association, is developing an integrated pest management program for Christmas tree growers. Selected growers will join in the effort by making regular observations on tree phenology,

weather conditions, and Cyclaneusma needlecast development. Growers will place spore traps within their plantations and monitor spore dispersal. This information, together with our research findings on various other Christmas tree pests, will be used to make integrated pest management decisions consistent with other management objectives.

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Literature Cited

1. Adams, G.C. 1988. Spore release and infection by *Cyclaneusma minus* needlecast disease of Scots pine in Michigan, p. 56-59. In: B.A. Montgomery (ed.). Michigan Forest Pest Report 1987. Mich. Coop. For. Pest Mgmt. Prog. Ann. Rep. 88-2.

2. Anderson, R.L., Skilling, D.D., Mosher, D.G. 1977. Naemacyclus needlecast of Scotch pine found in Michigan. Plant Dis. Rep. 61: 422.

3. Merrill, W., Kistler, B.R. 1974. Naemacyclus needlecast of Scots pine epidemic in Pennsylvania. Plant Dis. Rep. 58: 287-288.

4. Merrill, W., Robbins, K. 1987. How to identify and control Cyclaneusma needlecast of pines. USDA For. Serv. North Central For. Exp. Sta. HT-67. 6 p.

5. Prey, A.J. (ed.) 1985. Forest Pest Conditions in Wisconsin. Wisconsin Dept. of Natural Res., Ann. Rep. Madison, WI.

6. Wenner, N., Merrill, W. 1986. Cyclaneusma needlecast in Pennsylvania: a review. p. 35-40 In: G.W. Peterson (ed.). Recent Research on Conifer Needle Diseases. USDA Forest Service Gen. Tech. Rep. WO-5. 106 p.

7. Wenner, N., Merrill, W. 1988. *Cyclaneusma minus* infection controlled using Bravo 720 spray schedules. Phytopathology. 78: 1512.

Formation and Maturation of Apothecia of *Cyclaneusma Minus*[1,2,3]

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Abstract.–*Pinus sylvestris* needles infected with *Cyclaneusma minus* were collected at early stages of symptom development in mid-October and incubated at various temperatures for up to 12 weeks. Needles attained equal carrying capacities of about 237 apothecia/gram of oven-dry needle weight at all temperatures. Time to reach this carrying capacity was linearly and inversely correlated with temperature and ranged from 3 weeks at 19 C to 8 weeks at 2 C. The threshold temperature for apothecial development was calculated to be -3.8 C. Apothecia formed in nature during the fall and winter required a ripening period of warm temperatures for several weeks the following spring. During this ripening period the asci swelled and elongated above the ends of the paraphyses and then developed characteristic nipple-like swollen tips prior to liberating their ascospores in mid-April.

Introduction

Needlecast caused by *Cyclaneusma minus* (Butin) Dicosmo, Peredo & Minter is a major disease affecting production of Scots pine (*Pinus sylvestris* L.) Christmas trees in Pennsylvania (7). Although infection may occur nearly any time when temperatures are above freezing and moisture is available, the greatest proportion of infection in Pennsylvania occurs on first-year needles from late March to early June prior to their second season (7). Symptoms develop on second-year needles from late August through October followed by formation of apothecia from October through early December (7). If these fruiting bodies are examined in late fall or early winter, they appear to contain mature spores. That is, the spores are of mature size and two-septate. Although major spore releases may occur in early December (8), many apothecia bearing apparently mature ascospores liberate few if any spores when brought into the laboratory at that time, and the spores that are liberated often either do not germinate or lyse during germination (Merrill, Kistler & Zang, unpublished). In contrast, apothecia brought into the lab in mid-April release large numbers of ascospores that germinate readily. The following studies were done to examine the conditions required for formation and maturation of apothecia of *C. minus*.

Apothecial Formation: Materials and Methods

In mid-October, symptomatic second-year needles were picked from Scots pine Christmas trees in a commercial plantation in Clearfield County, PA. The needles, known from previous isolation studies to be infected, were still firmly attached to the twigs and had turned dusty yellow but had not yet developed the transverse brown bands characteristic of Cyclaneusma- infected needles. Preliminary studies had shown that the needles would need to be incubated at about 60% moisture content based on oven-dry (o.d.) weight to allow development of the pathogen. Needles stored at <50% moisture content appeared to be dehydrated and supported little fungal development. Needles stored at >70% moisture content were rapidly and profusely overgrown by saprophytic fungi.

No attempts were made to eliminate saprophytic fungi from the needle surfaces, as these organisms are part of the needle ecosystem within which the pathogen normally develops. The needles were thoroughly mixed and then soaked overnight in sterile distilled water, patted dry with paper towels, and finally air-dried on a lab bench for 75 min. This brought their moisture content to about 60% of o.d. weight. Twelve 40-g subsamples were each sealed in plastic bags which in turn were sealed in brown paper bags. These bags were placed into specially

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[2] Portions of these studies were conducted as part of U.S. Dept. Agr. Regional Research Project NE-27.

[3] Contribution No. 1483, Department of Plant Pathology, The Pennsylvania Agricultural Experiment Station.

[4] Professor, former Research Assistant, former undergraduate Plant Science student, and Senior Research Assistant, respectively, Department of Plant Pathology, The Pennsylvania State University. The first portion of this paper is based, in part, on an MS thesis of the second author. The second portion is based, in part, on a senior-year independent research project of the third author.

constructed mini-incubators (8), two bags per incubator. (The incubators were heated by light bulbs and the brown paper bags minimized temperature build-up within the plastic bags.) The incubators maintained temperatures of 2 ± 0.5, 5 ± 0.5, 9 ± 0.5, 11 ± 0.5, 16 ± 1, or 19 ± 1 C. Temperatures within the bags were monitored via thermocouples. The air within the bags was exchanged every 48 hr by opening them for 5 min.

Every 7 days 50 needles were removed from each bag and the number of apothecia that had developed sufficiently to rupture the needle epidermis was determined under 30X magnification. Each 50-needle subsample then was oven-dried to determine its moisture content, and the number of apothecia/g o.d. needle weight was calculated. When the needle moisture content of any bag dropped to about 50%, the needles were spread in a thin layer on a screen, misted with distilled water, and then rebagged. Throughout the experiment the mean needle moisture content of all bags, except in the 16 C chamber, ranged from 57.9 to 60.2%, a non-significant difference ($P > 0.05$). The 16 C chamber was an anomaly throughout the experiment, and data from it differed significantly from all other data. Because we were unable to account for the uncontrolled variations that existed within this one chamber, its data were excluded from the analyses.

Based on previous *in vitro* growth studies (2), we had postulated that the threshold temperature for apothecial development would lie within the range of temperatures selected. When this proved not to be true, the threshold temperature was calculated using the x-intercept method described by Arnold (1).

Apothecial Formation: Results

There was competition for needle substrate by an unidentified fungus that formed stromatic pycnidia on the needle surfaces. The fungus did not occur on needles incubated at 2 and 5 C. Although it occurred on needles at 9 and 11 C, it did not appear to limit growth and development of *C. minus* . The fungus was obvious at 16 C. At 19 C it was difficult to discern apothecia of *C. minus* after 9 weeks because of the profuse mycelial masses on the needle surfaces, and studies at this temperature were terminated.

Regardless of temperature, all needles reached a carrying capacity of about 237 apothecia/g o.d. weight. However, the time required to reach this carrying capacity varied with the temperature of incubation: 3 weeks at 19 C, 6 weeks at 11 and 9 C, 7 weeks at 5 C, and 8 weeks at 2 C (an error exists in fig. 10 of reference 8). The time-temperature regression was linear and highly significant ($R^2 = 0.81$, $P < 0.0001$). Interactions involving needle moisture content and replications were non-significant ($P > 0.05$). The calculated threshold temperature for apothecial development was -3.8 C.

Apothecial Maturation: Materials and Methods

On 1 March a bag (approximately 0.035 m^3) of symptomatic second-year Scots pine needles was collected from the plantation used in the first portion of this study. These needles were still loosely attached to the twigs and bore apothecia which had developed during the previous fall and/or winter. The needles were placed in nylon mesh envelopes on the ground under Scots pine Christmas trees at Penn State's Rock Springs Agricultural Research Center. With the onset of warming spring weather beginning on 17 March, 50 needles were selected at random each week. Rotorod® spore traps were run as described previously (7, 8). Twelve needles bearing multiple apothecia were selected. From 17 March to 21 April the apothecia were not fully opened; thus, their clypei were carefully cut away with a razor blade to expose the underlying hymenial layer. These needles were fixed in 5% glutaraldehyde (v:v) in 0.15 M cacodylate buffer (pH 7.2), dehydrated in a series of ethanol solutions ranging in concentration from 30 to 100%, critically point-dried with liquid CO_2, sputtered with gold to a thickness of 300 Å and examined at 10 KV using an I.S.I. (International Scientific Instruments, Santa Clara, CA) scanning electron microscope.

Apothecial Maturation: Results

On 24 March the hymenia of the apothecia, viewed from above, showed primarily the ends of paraphyses (fig. 1). Other apothecia collected at the same time and examined as squash mounts with light microscopy showed the asci contained mature-sized, two-septate ascospores. By 31 March the ends of some elongating asci were visible among the paraphyses (fig. 2). By 7 April the ends of some elongating asci protruded above the paraphyses and had begun to develop characteristic swollen tips (figs. 3 and 4). On 14 April a few asci had ejected their spores following a rain, as evidenced by open ascal pores (figs. 5 and 6) and confirmed by Rotorod® spore trapping, but the ends of many asci were still swelling prior to ascospore ejection. On 21 April virtually all visible asci possessed swollen tips (fig. 7). Following two days of drizzling rain on 26-27 April, nearly all asci in most apothecia had liberated their spores; the hymenia consisted only of paraphyses and appeared sponge-like due to spaces where the empty asci had collapsed (fig. 8). Squash mounts of similar apothecia indicated that these fruiting bodies were devoid of asci.

Discussion

Over 15 years of field observations have shown that in Pennsylvania the vast majority of Scots pine needles infected by *C. minus* develop symptoms from late summer

to mid-autumn of their second year. Apothecia develop on these needles from mid-autumn through early winter, and perhaps the following spring. It appears that the threshold temperature for apothecial production lies slightly below 0 C and the calculated value seems reasonable. Needles maintained at 2 C developed as many apothecia as needles maintained at 19 C, which is near optimum (2). This suggests that apothecial development may proceed throughout the winter during periods of above-freezing temperatures and perhaps under an insulating layer of snow at even colder temperatures.

Athough the ability of *C. minus* to develop apothecia at low temperatures may give it some competitive advantage over saprophytes, in this study the growth of saprophytic fungi on and in the needles had no discernable effect of the production of apothecia/g o.d. needle tissue. However, we were not able to determine the relative number, germinability, or vigor of ascospores produced in the presence of variable amounts of competing saprophytes.

Asci bearing what appear to be mature ascospores, that is, ascospores of "mature size" and possessing two septa, occur in apothecia that develop during the autumn, but often few such spores are liberated even when needles are placed in suitable environments in the lab. Our studies indicate that these asci require a "ripening" period of several weeks of warm weather the following spring. During this period the asci elongate and develop characteristic nipple-like swellings on their ends that later rupture, allowing ascospore ejection. During this ripening period some asci liberate their spores as evidenced by open ascal pores. Asci of this fungus mature successively (4).

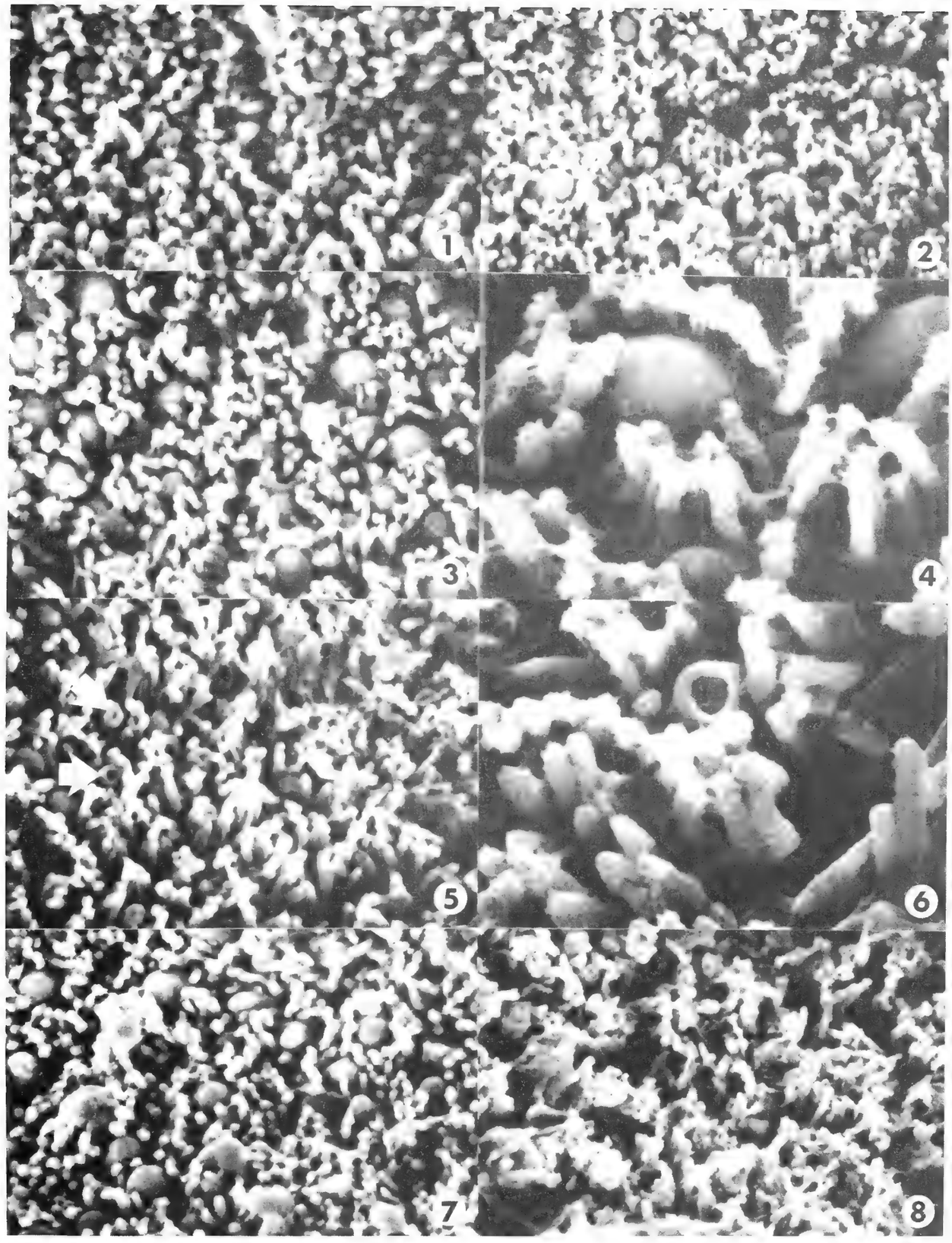
Extensive spore trapping, isolation and infection studies in Pennsylvania have shown that the ascospores of *C. minus* are liberated from mid- to late March throughout the growing season until cold weather terminates spore release sometime in November or December (6, 7, 8, Merrill, Zang & Wenner, unpublished). Some spore catches in the autumn or early winter may be as large as, if not larger than, those made during the peak periods of sporulation in the spring (8). Often these late season spore showers cause no infection, probably because environmental conditions favorable for infection either do not occur or are too brief (8, Merrill & Wenner, unpublished). But if spores are available throughout the growing season, why did the apothecia examined in the maturation portion of these studies liberate most of their ascospores at one time and then appear to be spent and incapable of further ascospore production? And why do some infected and cast needles lying on the duff appear to bear new and actively-sporulating fruiting bodies throughout May and early June?

We believe the answer lies in that we examined a particular cohort of needles from a single complement — needles that had been infected in the spring became symptomatic in the fall and supported apothecial formation in late fall and winter but still were attached to the twigs. It is probable that many of these needles had been infected at about the same time, and hence that the pathogen was in a similar developmental stage in most of these needles. Needles infected earlier may have already cast, and, lying on the duff, may have had sufficient moisture to support earlier maturation of the apothecia, which in turn may have accounted, in part, for some of the earlier spore showers encountered during spore trapping. [Spores have been collected from mid-March to early December (7, 8, Merrill & Wenner, unpublished).] In needles infected later in the growing season the fungus may not have developed sufficiently to form multiple apothecia and hence such needles would have been excluded from our study. These needles could have borne mature apothecia later in the spring, accounting for periodic spore catches throughout May and early June. Such spore showers would have gradually decreased in number and magnitude, as our spore trapping studies have shown (7, 8, Merrill & Wenner, unpublished).

Interpretation of all major studies of this pathogen has been hampered by the lack of definitive data on the length of the incubation period. That is, if a needle is infected in June, August or November of its first year, or March, April or May of the following year, when does it become symptomatic and when do apothecia develop on it? Our 15 years of field observations and isolation studies involving about 800,000 needles to date indicate that needles infected from March to May become symptomatic and bear apothecia in the fall and early winter of their second year. But a few needles become symptomatic from May through August of their second year and bear apothecia soon after. When are these needles infected? One can only postulate that they are infected early in their first growing season.

Available data suggest that a 3- to 4-month-long period of warm temperatures is required between infection and symptom development. This would account for the proven patterns of infection and the observed patterns of symptom development (7). But even although such a degree-day relationship may exist for the incubation period, this probably is not the complete story.

Many factors affect needle color and retention in *P. sylvestris* Christmas trees, including provenance and shearing practices (Merrill & Wenner, unpublished), insects (7), and possibly nutrition and other site factors. This confounds the interpretation of field observations and even prevents the accurate visual assessment of fungicidal spray trials. Rack and Scheidemann (5) concluded that in Germany *C. minus* is an endophyte, that is, a nonpathogenic needle inhabitant that appears only when the needles age or become stressed. They also concluded that *C. minus* was more or less secondary to needlecast caused by *Lophodermium seditiosum* Minter, Staley & Millar (5). We provided some data supporting the endophyte theory over a decade ago (3). However, in most plantations where we have worked with Cyclaneumsa



Figures 1 through 8.—Scanning electron microscope views of developing apothecial hymenia of *Cyclaneusma minus*. **Figure 1.**—On 24 March hymenia consisted primarily of paraphyses; the ends of a few elongating asci could be seen among the paraphyses. 1700X. **Figure 2.**—On 31 March numerous asci had elongated sufficiently to be visible among the paraphyses. 1700X. **Figure 3.**—By 7 April the asci had elongated to protrude above the ends of the paraphyses and had developed nipple-like swellings on their tips. 1700X. **Figure 4.**—Nipple-like swellings on ascus tips. 5200X. **Figure 5.**—By 14 April a few asci had sufficiently matured to release ascospores after a light rain, as evidenced by open ascal pores (arrows). 1700X. **Figure 6.**—Open ascal pore. 5900X. **Figure 7.**—By 21 April most remaining asci had elongated above the ends of the paraphyses and had developed swollen tips. 1700X. **Figure 8.**—Following two days of rain, on 27 April all asci had released their spores and collapsed, leaving a sponge-like hymenium composed of paraphyses and the cavities previously occupied by asci. 1700X.

needlecast for the past 15 years, Lophodermium needlecast does not occur. Indeed, the timing of infection and casting in Lophodermium needlecast in Pennsylvania (July-September of the first year and March-May the second year, respectively) would virtually eliminate Cyclaneusma needlecast from the plantations. The generally low levels of infection by *C. minus* from June to September of the first year would be masked and eliminated by the concurrent high levels of infection by *L. seditiosum*. Needles would be dying and casting due to Lophodermium needlecast prior to or concurrent with the major infection period of *C. minus* . Furthermore, the extensive needle losses associated with Cyclaneusma needlecast that occur annually in spite of great year-to-year variations in climate not only in the absence of other foliar pathogens but also with no demonstrated effect (thus far) of site quality, together with the major differences in susceptibility of various *P. sylvestris* provenances to the fungus with their associated effects on symptom development (7) cause us to doubt that *C. minus* is an endophyte. But many key questions are still unanswered and Cyclaneusma needlecast remains an intriguing and enigmatic problem.

Literature Cited

1. Arnold, C.Y. 1959. The determination and significance of the base temperature in a linear heat unit system. Proc. Am. Soc. Hort. Sci. 74:430-435.
2. Kistler, B.R. 1977. Etiology, symptomology, epidemiology, and chemical control of Naemacyclus needlecast. M.S. thesis, The Pennsylvania State Univ., University Park. 27 p.
3. Kistler, B.R., Merrill, W. 1978. *Naemacyclus minor* : high levels of infection in symptomless Scots pine. (Abstr.) Phytopathology News 12:69-70.
4. Minter, D.W., Cannon, P.F. 1984. Ascospore discharge in some members of the Rhytismataceae. Trans. Brit. Mycol. Soc. 83:65-92.
5. Rack, K., Scheidemann, U. 1987. Über Sukzession und pathogene Eigenshaften Kiefernnadeln bewohnender Pilze. Eur. J. For. Path. 17:102-109.
6. Wenner, N.G. 1987. The effect of chlorothalonil on the infection of Scots pine Christmas trees. M.S. thesis, The Pennsylvania State University, University Park. 19 p.
7. Wenner, N.G., Merrill, W. 1986. Cyclaneusma needlecast of Scots pine in Pennsylvania: a review. p. 35-40. In: G.W. Peterson (ed.). Recent Research on Conifer Needle Diseases. USDA For. Serv. Gen. Tech. Rep. WO-50. 106 p.
8. Zang, L.E. 1984. Spore release and apothecial development in *Naemacyclus minor* Butin. M.S. thesis, The Pennsylvania StateUniv., University Park. 49 p.

Control of Cyclaneusma Needlecast on Scots Pine in Pennsylvania[1,2]

N.G. Wenner and W. Merrill[3]

Abstract.–Three rates of a flowable formulation of chlorothalonil were evaluated from 1984 through 1988 to determine the minimum number and best timing of applications to prevent *Cyclaneusma minus* infection of *Pinus sylvestris*. Studies were conducted in three commercial Christmas tree plantations in Clearfield County, Pennsylvania, using the Pike Lake strain of *P. sylvestris*. Chlorothalonil rates of 2.3, 4.7, and 9.3 kg a.i./ha (kg active ingredient per ha) were applied by backpack mist blower. Treatments were evaluated through direct isolation of the fungus from needles to determine the percentage of infected needles. Five 2.3 kg a.i./ha sprays applied in June, August, October, and the following March and May provided excellent continuous protection.

Introduction

In 1972, *Cyclaneusma minus* (Butin) DiCosmo, Peredo, & Minter (=*Naemacyclus minor* Butin)[Ascomycetes: Rhytismatales](2) was discovered causing needlecast of Scots pine (*Pinus sylvestris* L.) Christmas trees in Pennsylvania (10). This fungus is distributed nearly worldwide and attacks many species of hard pines, from seedlings to mature trees. It occurs throughout the northeastern United States in plantations, landscape plantings, and wild or abandoned stands of *P. mugo* Turra., *P. nigra* Arnold, *P. ponderosa* Laws., *P. sylvestris*, and *P. virginiana* Mill.(19). Infected one-year-old and older needles turn prematurely dusty-yellow with transverse brown bars. White- to cream-colored apothecia later develop within the brown bars and these needles may cast or remain hanging in the tree (7). Severely affected trees retain only current-year needles, appear thin and ragged, and are greatly devalued or completely unmarketable as Christmas trees or landscape ornamentals. Losses in Christmas tree plantations are especially severe because the tightly sheared, closely spaced trees present an ideal microclimate for fungal development. Some researchers question the pathogenicity of *C. minus*, particularly when other foliar pathogens are present (16). However, severe Cyclaneusma needlecast occurs in Pennsylvania plantations in the absence of other foliar diseases or foliage feeding insects (19).

Scots pine is a popular and widely grown Christmas tree because of its rapid growth and tolerance of poor sites. In the early 1970's Scots pine comprised about 70% of Pennsylvania Christmas trees. In the 1980's, however, its production dropped to less than 36% (4) due, in part, to the inability to economically control Cyclaneusma needlecast. Individual resistant trees occur in virtually every Scots pine seed source, but breeding or cloning of such trees has not yet been undertaken. Certain seed sources have larger proportions of resistant individuals than others (11), but also may lack good needle color, hardiness, or other desirable growth characteristics.

Cyclaneusma minus has a prolonged incubation period typical of needlecast fungi, but also resembles needle blight fungi because it can infect needles of any age whenever temperatures are above 2 C and free moisture is present (21). In Pennsylvania four overlapping infection periods have been identified, resulting in nearly year-round infection (9, 13). This combination of year-round infection and continuously susceptible needles requires preventative spray protection for Christmas trees.

Many chemical formulations have been tested for the control of Cyclaneusma needlecast including: anilazine (1, 5), Bay Meb 6447 (6), benomyl (1, 3, 5, 7), Bordeaux mixture (Glenn Peterson, 1975 pers. comm.), BP crop oil (5), captafol (20), carbendazim (1, 5), copper oxychloride (5), Curitan (3), diclone (5), Dithane M-45 (8), dodine (1, 3, 5), dyrene (3), L-argenine monohydrochloride (5), mancozeb (12), Manzate 200 (7), Orthocide (8), orthophenylenediamine (5), oxycarboxin (5), and Zyban (14). However, most effective compounds are active for only 10-14 days. Repeated application of such short-lived compounds is economically impractical. In 1982-83 the Daconil 2787F flowable formulation of chlorothalonil showed unexpected longevity in preventing *C.*

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[2] Contribution No. 1776, Department of Plant Pathology, The Pennsylvania Agricultural Experiment Station.

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minus infection. Applications at 3-week intervals from May through October 1982 using 4.7 kg a.i./ha (kg active ingredient per ha) were still providing complete protection 10 months later, in August 1983 (18). Trees in adjacent check blocks and border rows incurred an average 48% increase in the level of infection during that time, indicating that elimination of inoculum from the plot was not involved. These studies provided the first possibility of developing an economically feasible spray schedule for the control of Cyclaneusma needlecast.

Materials and Methods

Three sequential studies were conducted to determine the most effective rates and spray timing to prevent *C. minus* infection using flowable chlorothalonil formulations (Bravo 500 and Bravo 720, Fermenta ASC Corporation, Mentor, Ohio). Commercial Christmas tree plantations in Clearfield County, Pennsylvania were selected where Cyclaneusma needlecast had been prevalent for many years, but no other significant pests were present. The trees in the plantation were 1-2 m Scots pine of the Pike Lake strain (Noecker Pines, Dorr, Michigan) which is moderately susceptible to Cyclaneusma needlecast. The trees were planted at 1.5 x 1.8 m spacing, and two rows of trees were left between treatment blocks to minimize the effects of spray drift. Fungicide applications were made with a Solo backpack mist blower (Solo Inc., Newport News, Virginia) at the rate of 153 liters of spray mix/ha.

Each treatment block of 18 to 30 trees contained ten permanently numbered and labeled 'sample' trees. Efficacy was determined through direct isolation of the fungus from host needles. On each sampling date, four twigs were cut, one in each cardinal direction, at 0.5 m from the ground from each of the ten sample trees per block. From each twig, ten needles were randomly removed, thus yielding 40 needles per tree, and a total of 400 needles per treatment block. Needles were surface sterilized in 0.52% aqueous sodium hypochlorite containing 0.75 ml/l "Joy" ® (Proctor and Gamble, Cincinnati, Ohio) dish detergent. Needles were cut into three pieces and pushed slightly into the surface of 2% acidified malt agar (AMA = 20.0 g Difco malt extract, 15.0 g Difco flake agar, 1.0 liter double-distilled water, 1.0 ml of 88.3% lactic acid added after autoclaving). The plates were incubated in diffuse light at 21 C for 21 days. Infected needles were identified by the presence of the distinctive whitish Cyclaneusma colonies growing from the cut needles. In this manner, disease progress curves for a single complement of needles were determined for each of the ten sample trees per treatment block

Study 1 determined the length of protection provided by the Bravo 500 formulation at rates of 2.3, 4.7, or 9.3 kg a.i./ha applied at various intervals between June 1984 and July 1985 (tables 1 and 2). Check trees were sampled every 3 to 4 weeks; sprayed trees were sampled periodically during the fall and spring infection periods and at the end of the study. A total of 22,800 needle isolations were made.

Table 1.—Percentages of the 1984 complement of *Pinus sylvestris* needles infected by *Cyclaneusma minus* following various treatments with chlorothalonil.

		Sampling Dates									
		1984					1985				
Treatment	Block	14/6	28/6	19/7	9/8	5/11	24/1	9/4	9/5	6/6	12/7
2.3 kg a.i./ha											
14/6/84	1	—	—	(0.5)[a]	—	(5.0)	21.5	56.5	—	—	—
14/6, 9/8/84	4	—	—	—	—	—	7.8	32.3	—	—	92.3
14/6, 9/8, 5/11/84	6	—	—	—	—	—	1.8	12.3	—	56.5	67.8
14/6, 9/8, 5/11/84 and 9/4/85	7	—	—	—	—	—	6.5	14.0	14.0	—	26.5
4.7 kg a.i./ha											
14/6/84	3	—	—	(0)	—	(4.5)	21.0	44.8	—	—	75.8
14/6, 5/11/84	11	—	—	—	—	—	10.8	35.0	—	—	64.5
14/6, 5/11/84 and 9/4/85	8	—	—	—	—	—	15.5	35.5	35.5	—	35.5
9.3 kg a.i./ha											
14/6/84	9	—	—	(1.0)	—	(0.5)	7.0	19.5	—	77.5	87.3
Untreated Checks[b]	2,5,10	1.3	2.3	2.5	4.0	6.7	28.5	63.7	71.0	88.9	88.9

[a] (#) - isolations made from 5/10 reps in block.

[b] average of three checks.

Table 2.—Area Under the Disease Progress Curve (AUDPC) values for the percentages of the 1984 needle complement of *Pinus sylvestris* infected[a] by *Cyclaneusma minus* following various treatments with chlorothalonil

Block	Treatment kg a.i./ha	1984 Application Date	AUDPC[b]
2,5,10	0	No sprays	1400.9 A
1	2.3	14 June	1185.6 AB
3	4.7	14 June	999.4 BC
8,11	4.7	14 June, 5 Nov	746.7 CD
4	2.3	14 June, 8 Aug	604.2 DE
9	9.3	14 June	402.8 EF
6,7	2.3	14 June, 8 Aug, 5 Nov	262.2 F

[a]Includes isolation data through 9 April 1985.

[b]Values followed by the same letter are not significantly different (P=0.05) using the Duncan's multiple range test.

Table 3.—Percentages of the 1985 complement of *Pinus sylvestris* needles infected by *Cyclaneusma minus* following various treatments with chlorothalonil

Treatment	Block	Percentage of Needles Infected Date: 5/3/86	24/3/86	12/6/86
2.3 kg a.i./ha 6/6, 20/8, 9/10/85, and 24/3/86	1	3.0	3.0	48.0
9.3 kg a.i./ha 6/6/85 + 4.7 kg a.i./ha 24/3/86	3	7.0	7.0	23.0
Untreated Check	2	11.0	15.8	67.0

Study 2, conducted from June 1985 through June 1986, replicated the Bravo 500 summer sprays from the two best treatments in Study 1, but applied the fall and spring applications earlier (table 3). Sampling was done prior to and during the spring infection period and at the end of the study. Isolations were made from 3600 needles.

In study 3, (Bravo 720 formulation was used) conducted from June 1987 through June 1988, the timing of the summer and fall applications of Study 2 was replicated, and a second spring application was added in May (table 4). Check trees were sampled every 3 to 4 weeks. Sprayed trees were sampled at the beginning and end of the study and before each pesticide application for a total of 11,600 needle isolations.

Table 4.—Percentages of the 1987 complements of *Pinus sylvestris* needles infected by *Cyclaneusma minus* following various treatments with chlorothalonil

Treatment	% Needles Infected on Selected Sampling Dates* 1987 8/6	18/8	14/10	7/12	1988 2/2	23/3	10/5	10/6
Unsprayed Check	3.0 aA	9.5 bA	22.5bcA	29.5 cd	29.8 cd	37.5 dA	61.0 eA	87.8 fA
chlorothalonil 2.3 kg a.i./ha (8/6, 18/8, 14/10/87, and 23/3, 10/5/88)	3.0 aA	3.0 aB	3.0 aB	—	—	3.0 aB	3.0 aB	3.0 aB
chlorothalonil 9.3 kg a.i./ha (8/6/87) and 2.3 kg a.i./ha (10/5/88)	3.0 aA	3.0 aB	3.0 aB	—	—	3.5 aB	24.0 bC	24.0 bC

*Numbers in rows followed by the same lower case letter are not significantly different. Student's t, P = 0.05.

Numbers in columns followed by the same upper case letter are not significantly different. Student's t, P = 0.05.

Results and Discussion

Study 1.—The interval of fungicide protection provided by the summer and fall applications is best evaluated separately from that of the spring application. The efficacy of the summer and fall applications is indicated by the percentage of infected needles on 24 January and 9 April (table 1, figures 1, 2, and 3). Proportions of infected needles among the three check plots did not differ significantly ($P > 0.05$); thus, their average was used in all analyses.

Among the 2.3 kg a.i./ha treatments (table 1, figure 1), the effect of the 14 June 1984 single spray (block 1) was negligible. By 24 January 1985 the level of infection had reached 21.5% and was not significantly different from that of the check and this portion of the study was terminated. All multiple 2.3 kg a.i. l/ha treatments (blocks 4, 6, 7) held levels of infection significantly lower than that of the check through 24 January and 9 April ($P < 0.001$). The two sprays applied to block 4 on 14 June and 9 August held the level of infection to 7.8% through 24 January 1985. Beyond that, however, the level of infection increased significantly, eventually equalling that of the check by 12 July. Blocks 6 and 7 received sprays on 14 June, 9 August, and 5 November and maintained levels of infection which were not significantly different

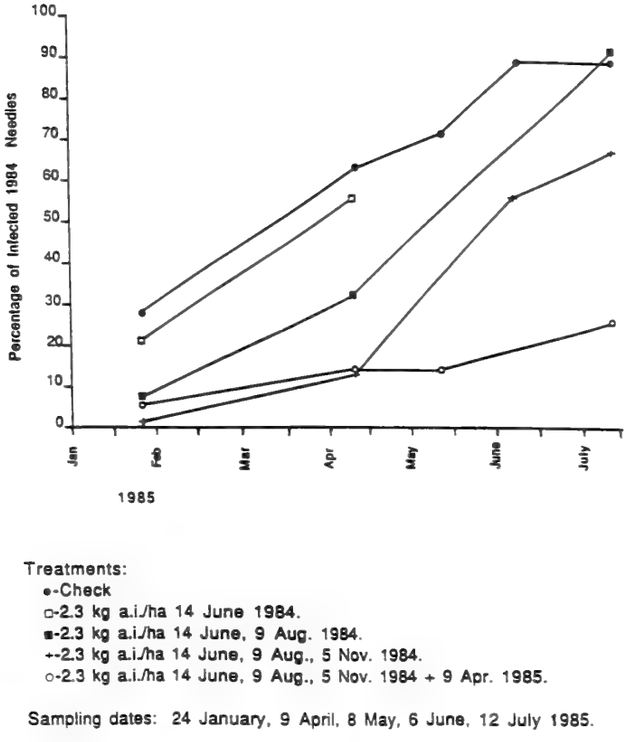


Figure 1.—Percentages of the 1984 complement of *Pinus sylvestris* needles infected by *Cyclaneusma minus* following various treatments with chlorothalonil at 2.3 kg a.i./ha during 1984/85.

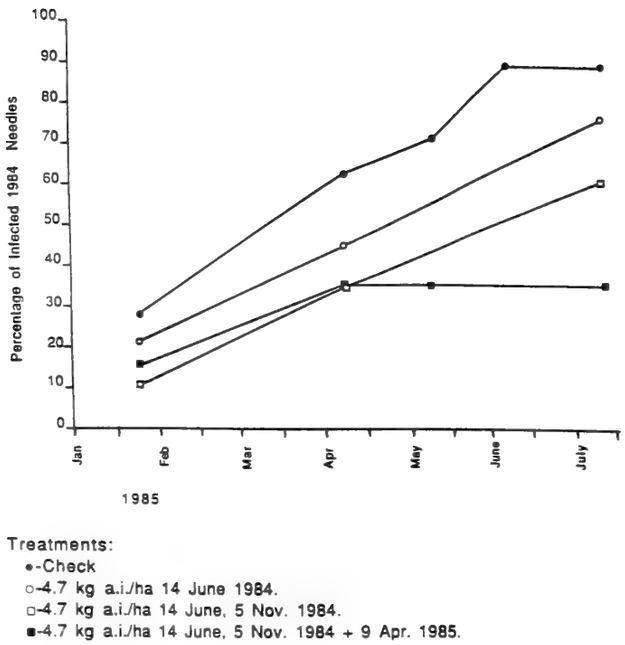


Figure 2.—Percentages of the 1984 complement of *Pinus sylvestris* needles infected by *Cyclaneusma minus* following various treatments with chlorothalonil at 4.7 kg a.i./ha during 1984/85.

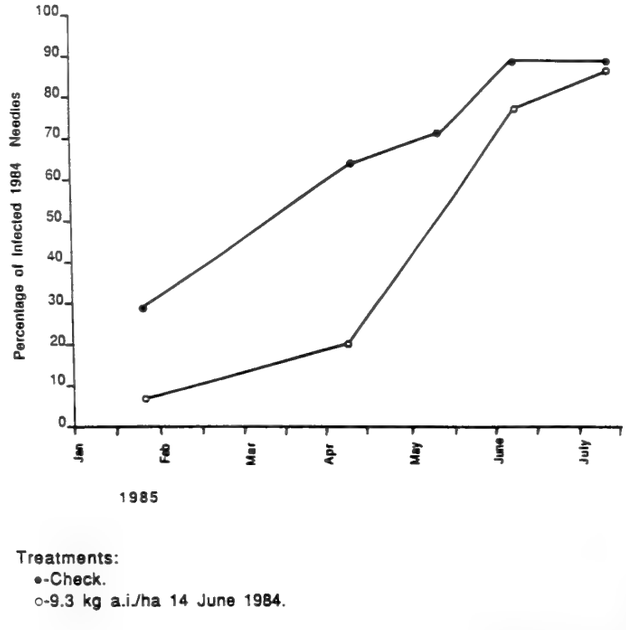


Figure 3.—Percentages of the 1984 complement of *Pinus sylvestris* needles infected by *Cyclaneusma minus* following treatment with chlorothalonil at 9.3 kg a.i./ha during 1984/85.

from each other on 24 January (1.8% and 6.5%, respectively) and 9 April 1985 (12.3% and 14.0%, respectively). Beyond 9 April the level of infection in block 6 continued to increase significantly. The single 2.3 kg a.i./ha rate spring spray applied to block 7 on 9 April held the 14% level of infection through 8 May. Beyond that the level of infection increased significantly to 26.5% by the end of the study on 12 July. The latter increase in the level of infection in block 7 probably occurred near the end of the study in late June and early July. In a commercial plantation, an early to mid-June spray would have been applied to protect the new complement of needles, thus preventing further infection.

Results using the single 4.7 kg a.i./ha spray (table 1, figure 2) applied on 14 June (block 3) were nearly identical (21.0%) to that of the single 2.3 kg a.i./ha application on 14 June in block 1 (21.5%). Similarly, the multiple 4.7 kg a.i./ha treatments applied on days 14 June and 5 November (blocks 8 & 11) were not better (15.5% & 10.8%, respectively) than the multiple 2.3 kg a.i./ha application on 14 June and 9 August (7.8% - block 4).

Between 24 January and 9 April the multiple 4.7 kg a.i./ha treatment blocks incurred significant increases in infection as did the 2.3 kg a.i./ha treatment blocks (table 1, figure 2). Beyond 9 April the level of infection in block 11 continued to increase significantly. However, the additional 4.7 kg a.i./ha application on 9 April held infection to 35% in block 8 through the end of the study.

The single 9.3 kg a.i./ha application (block 9) on 14 June held infection to 7.0% through 24 January and 19.5% through 9 April 1985 (table 1, figure 3). Although the 12.5% increase between 24 January and 9 April was significant, it was still significantly less than that of the check (35.2%) during that time. Beyond 9 April the level of infection increased sharply, and eventually equalled that of the check by the end of the study.

The 2.3 kg a.i./ha treatments provided approximately 2 months of protection. Doubling the fungicide rate to 4.7 kg a.i./ha did not yield significantly better protection or permit fewer applications. Assuming that the 2.3 kg a.i./ha treatments on 14 June and 9 August each provided about 2 months protection, the infection in those blocks (4, 6, and 7) probably occurred during mid- to late October. This increase in infection might have been prevented if the fall spray date had been somewhat earlier than 5 November.

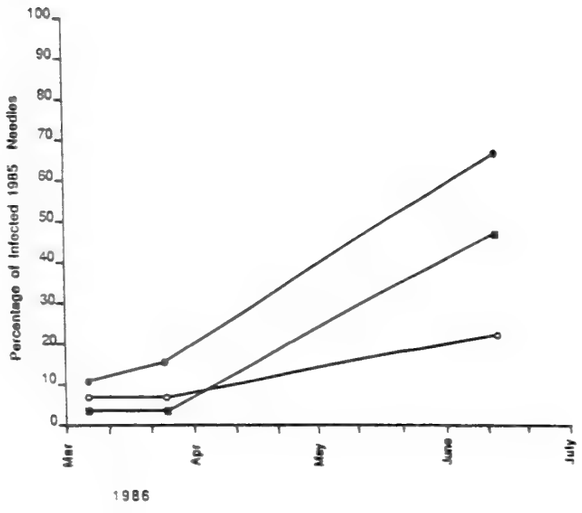
The significant increase in the level of infection in all blocks between 24 January and 9 April occurred as the weather warmed in late March and early April, and the spring infection period began. The significant increases in infection prior to 9 April indicated that the spring application should have been applied earlier. Comparing the two spring application rates [blocks 7 (2.3 kg a.i./ha) versus block 8 (4.7 kg a.i./ha)], the 4.7 kg a.i./ha rate provided significantly better protection from 9 April through the end of the study than the 2.3 kg a.i./ha rate (0.0% increase in the level of infection versus 12.5%, respectively).

The Study 1 treatments also were evaluated by calculating the area under the disease progress curve (AUDPC) (17) for each of the ten sample trees per block through 9 April (table 2). These ten AUDPC values were then averaged to produce the block AUDPC. The AUDPC block values were statistically separated with Duncan's New Multiple Range test. These data indicated the best summer and fall treatments as those having the lowest AUDPC values. Data from blocks 6 and 7 were averaged, as were data from blocks 8 and 11, since these treatments were identical until 9 April. The two best treatments were the 2.3 kg a.i./ha applications in June, August and November (blocks 6 and 7) and the single 9.3 kg a.i./ha application in June (block 9). Thus, these two treatments were selected as bases for further modification and evaluation in Study 2.

Study 2.—In Study 2, from June 1985-June 1986, 2.3 kg a.i./ha treatments were applied in June and August as they were in Study 1. However, the fall application was moved from November to October to provide more protection during the fall infection period, and the first spring spray also was applied earlier. Thus, four applications at 2.3 kg a.i./ha were made: 6 June, 20 August, 9 October 1985, and 24 March 1986. The single 6 June 1985 application at 9.3 kg a.i./ha was followed by an application at 4.7 kg a.i./ha on 24 March 1986. The fungicide application, sampling, and isolations were carried out as in Study 1. Results are shown in table 3.

The levels of infection were lower than usual in 1985-86. Unsprayed trees averaged 11.0% and 15.8% infection on 5 March and 24 March 1986, respectively, and by the end of the study (12 June 1986) had 67.0% infection. Both treatments, however, held levels of infection significantly lower than those of the check on all sample dates.

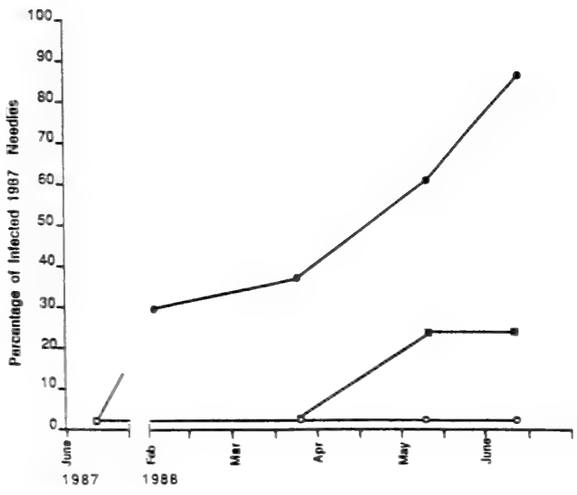
The results of Study 2 (table 3, figure 4) confirmed that either one treatment at 9.3 kg a.i./ha or three treatments at 2.3 kg a.i./ha provided excellent protection from *C. minus* infection during the summer and fall. On 24 March, when the spring spray was applied, the levels of infection in the two treatments were not significantly different, 3.0% and 7.0% ($P > 0.05$), respectively. However, neither the 2.3 kg a.i./ha nor the 4.7 kg a.i./ha spring spray provided adequate protection during the spring infection period. Between 24 March and the end of the study on 12 June, levels of infection in both treatments increased significantly. The level of infection in the 2.3 kg a.i./ha spring spray treatment increased to 48.0%. The level of infection in the trees receiving the 4.7 kg a.i./ha rate spring spray treatment increased to 23.0%. These levels of infection were significantly different and unacceptably high. In Study 1, the 2.3 kg a.i./ha summer and fall applications provided approximately 2 months protection. However, this interval is probably reduced



Treatments:
•-Check
○-2.3 kg a.i./ha 6 June, 20 Aug., 9 Oct. 1985 + 24 March 1986.
▪-9.3 kg a.i./ha 6 June 1985 + 4.7 kg a.i./ha 24 March1986.

Sampling dates: 5 March, 24 March, 12 June 1986.

Figure 4.—Percentages of the 1985 complement of *Pinus sylvestris* needles infected by *Cyclaneusma minus* following various treatments with chlorothalonil during 1985/86.



Treatments:
•-Check
○-2.3 kg a.i./ha 8 June, 18 Aug., 14 Oct. 1987 + 23 March, 10 May 1988.
▪-9.3 kg a.i./ha 8 June 1987 + 2.3 kg a.i./ha 10 May 1988.

Sampling dates: 8 June, 18 Aug., 14 Oct., 7 Dec. 1987, and 2 Feb., 23 March, 10 May, 10 June 1988.

Figure 5.—Percentages of the 1987 complement of *Pinus sylvestris* needles infected by *Cyclaneusma minus* following various treatments with chlorothalonil during 1987/88.

during the spring infection period when warm temperatures, frequent spring rains, and high inoculum pressure are common. The 4.7 kg a.i./ha spring spray in Study 1 maintained protection from 9 April through 12 July. In Study 2, this spray was applied earlier, on 24 March, and the chemical probably deteriorated during May when inoculum pressure peaked and protection was crucial. Thus, Study 2 treatments showed that one spring application at either 2.3 or 4.7 kg a.i./ha was not sufficient to provide protection during the spring infection period.

Study 3.—In Study 2, either 3 sprays at 2.3 kg a.i./ha applied in June, August and October, or a single application at 9.4 kg a.i./ha in June prevented infection throughout the summer, fall, and winter. Study 3 replicated the summer and fall spray applications of Study 2, but evaluated the addition of a second 2.3 kg a.i./ha spring application to control the spring infection period. Thus, five 2.3 kg a.i./ha rate sprays were applied in Study 3 — June, August, and October 1987, and March and May, 1988 (table 4). The single 9.3 kg a.i./ha application also was made in June 1987, was followed with a single spring application (May 1988) at 2.3 kg a.i./ha.

The results (table 4, figure 5) verified the effectiveness of either summer/fall treatment. The level of infection as of 23 March 1988 was 3.0% in the multiple 2.3 kg a.i./ha treatment block, which was not significantly different from 7.0% infection in the single 9.3 kg a.i./ha treatment block. The level of infection in the checks (37.5%) was significantly higher than the treatments on that date. The two 2.3 kg a.i./ha spring applications provided excellent protection, maintaining the 3.0% level of infection through the end of the study. Although the single 9.3 kg a.i./ha rate application provided excellent summer/fall protection, a significant increase in the level of infection (to 24.0%) occurred between 23 March and 10 May before the single spring 2.3 kg a.i./ha spray was applied. Thus, Study 3 demonstrated again that two applications were necessary for complete protection during the spring infection period.

The results of these three studies from 1984-1988 showed that infection by *C. minus* infection was prevented by flowable chlorothalonil products. A 2.3 kg a.i./ha rate application (Bravo 720) provided about 2 months protection during the summer and fall infection periods. This interval of protection was reduced during the spring infection period when inoculum pressure peaked (13, 21) and frequent rains provided prolonged periods favorable for infection. Thus, in Pennsylvania, two fungicide applications at the 2.3 kg a.i./ha rate are necessary to prevent infection from March through early June. Five sprays at 2.3 kg a.i./ha applied in June, August, and October, and the following year in March and May, provided year-round protection. In timing the first spring application, we have had good results by applying the spray when the buds on purple lilacs (*Syringa vulgaris* L.) in the area have swollen so that 1.5-3.5 mm of new green tissue has expanded beyond the tan bud scale.

Doubling the fungicide rate did not provide significantly better protection. A single 9.3 kg a.i./ha application in June provided good protection during the summer,

fall, and winter, but would require two 2.3 kg a.i./ha applications the following March and May to be as effective as the five-spray 2.3 kg a.i./ha treatment. Although the single June 9.3 kg a.i./ha treatment could save the labor and equipment costs for the August/October applications, many growers spray for other pests at these times anyway. The high rate used for the single summer/fall spray also uses 2.4 kg a.i./ha more fungicide than required in the 5-spray schedule. Further, unusually warm, wet fall weather could hasten the degradation of a single 9.3 kg a.i./ha June spray and infection could result during November or early December. Consequently, the five-spray (March, May, June, August, October) 2.3 kg a.i./ha treatment is recommended. Integrated with other pest control schedules, these treatments may be used economically and effectively. On the basis of these and other studies, chlorothalonil (Bravo 500 and 720) was labelled by the Environmental Protection Agency for the control of Cyclaneusma needlecast in August 1987. Compared to previous chlorothalonil evaluations controlling *C.minus* infection (15), the five-spray schedule reduces total fungicide usage in terms of active ingredient (a.i.) per hectare by 65%- from 32.9 to 11.4 kg a.i./ha. Commercial growers have field tested the five-spray schedule with excellent results. In addition, a Wisconsin grower reported that this spray treatment also controlled brown spot needle blight caused by *Mycosphaerella dearnessii* Barr (Lorin Zastoupil, pers. comm.). The timing of the sprays should also provide adequate protection for needlecast caused by *Lophodermium seditiosum* Minter, Staley & Millar.

Literature Cited

1. Anon. 1979. Rept. Forest Res. Inst. New Zealand, 1978. Wellington, N.Z. 112 p.
2. DiCosmo, F., Peredo, H. , Minter, D.W. 1983. *Cyclaneusma* gen. nov., *Naemacyclus* and *Lasiostictis*, a nomenclatural problem resolved. Eur. J. For. Path. 13:206-212.
3. Gadgil, P.D. 1977. How important is Naemacyclus? What's New in Forest Research No. 56. N. Z. Forest Res. Inst., Rotorua, N.Z. 4 p.
4. Grace, J. R. 1985. Economic and production survey, Pennsylvania Christmas tree growers, 1985, preliminary results. The Pennsylvania State Univ., School For. Resources, University Park, unpublished report, 7 p.
5. Hood, I.A., Vanner, A.L. 1984. Cyclaneusma (Naemacyclus) needlecast of *Pinus radiata* in New Zealand. 4: Chemical control research. N. Z. For. Sci. 14:223-228.
6. Kistler, B.R., Merrill, W. 1978. Testing Bay Meb 6447 for systemic control of Naemacyclus needlecast of Scots pine, 1977. Am. Phytopath. Soc., Fungicide & Nematicide Tests 35:131.
7. Kistler, B.R., Merrill, W. 1978. Etiology, symptomology, epidemiology and control of Naemacyclus needlecast of Scotch pine. Phytopathology 68:267-271.
8. Magnani, G. 1972. Sulla presenza di *Naemacyclus niveus* su aghi di *Pinus radiata*. Pubbl. Centro Sper. Agric. For. 11:315-320.
9. Merrill, W. 1982. Fourth infection period in Naemacyclus needlecast of Scots pine. (Abstr.) Phytopathology 72:264.
10. Merrill, W., Kistler, B. R. 1974. Naemacyclus needlecast of Scots pine epidemic in Pennsylvania. Plant Dis. Rep. 58:287-288.
11. Merrill, W., Slover, S. 1983. Naemacyclus needlecast resistance of twelve Scots pine seed sources. Am. Christmas Tree J. 27(3):33-34.
12. Merrill, W., Kistler, B. R., Bowen, K. 1980. Chemical control of Naemacyclus needlecast of Scots pine. (Abstr.) Phytopathology 70:466.
13. Merrill, W., Kistler, B. R., Zang, L., Bowen, K. 1980. Infection periods in Naemacyclus needlecast of Scots pine. Plant Dis. 64:759-761.
14. Merrill, W., Sninsky, M. R., Green, N. 1982. Fungicide evaluation for control of Naemacyclus needlecast, 1981. Am. Phytopath. Soc., Fungicide & Nematicide Tests 37:139-140.
15. Merrill, W., Wenner, N., Wang, L. 1984. An experimental fungicide/insecticide spray schedule for Scots pine. Pa. Christmas Tree Growers' Assoc. Bull. 163:8.
16. Rack, K., Scheidemann, U. 1987 Über Sukzession und pathogene Eigenschaften Kiefernnadeln bewohnender Pilze. Eur. J. For. Path. 17:102-109.
17. Tooley, P.W., Grau, C.R. 1984. Field characterization of rate-reducing resistance to *Phytophthora megasperma* f. sp. *glycinea* in soybean. Phytopathology 74:1201-1208.
18. Wenner, N., Merrill, W. 1984. Evaluation of Daconil 2787F to control Naemacyclus needlecast, 1983. Am. Phytopath. Soc., Fungicide & Nematicide Tests 39:197.
19. Wenner, N.G., Merrill, W. 1986. Cyclaneusma needlecast of Scots pine in Pennsylvania: a review. p. 35-40. In: G.W. Peterson (ed.). Recent Research on Conifer Needle Diseases. USDA For. Serv. Gen. Tech. Rep. WO-50, 106 p.
20. Zang, L., Merrill, W. 1980. Control of *Naemacyclus minor* needlecast with Difolatan. (Abstr.) Phytopathology 70:470.
21. Zang, L.E. 1984. Spore release and apothecial development in *Naemacyclus minor* Butin. M.S. thesis, The Pennsylvania State Univ., University Park. 49 p.

Root Cold Stress Causing a Premature Yellowing of Oldest Scots Pine Needles[1]

Risto Jalkanen[2]

Abstract.—The sudden loss of the oldest needles of Scots pine (*Pinus sylvestris* L.) at the height of the 1987 growing season on the poorest forest site classes in SE Lapland, northern Finland, was postulated to be caused by very cold (<-40 C) weather during the snowless December of 1986 which injured the root systems. In a 1988 pilot study, temperatures of the main root zone in a snowless plot fell below the tolerance of root systems to survive without injuries. Delayed thawing of an artificially produced ice layer and ground frost in the spring following a snowless period in winter produced similar loss of the oldest complements of needles at the same time in early July as had happened naturally a year before. Injuries to the root system led to deficiencies in water supply and nutrition, which were reflected as reduced growth of pine as early as in the year of natural defoliation. The rapid recovery of Scots pine in forests a year after the needle loss indicated an acute effect of climatical extremes rather than continuous effects of air pollutants; e.g., ozone, as a cause of needle loss.

Sudden Needle Loss in 1987

At the height of the 1987 growing season, the oldest needles of Scots pine (*Pinus sylvestris* L.) started to turn yellow and then shed in one month, clearly before the end of the summer. Needle loss was most severe on the poorest dry pine heaths. These sites were characterized by fine-textured sandy soil, a thin humus layer and a very heavily browsed *Cladonia* layer. The main region of needle loss was restricted primarily to latitudes between 65 N and 67 N in southern Lapland and northern Ostrobothnia. Needle loss did not occur in corresponding sites in other parts of Lapland.

From one to five, averaging approximately 2.4, needle complements were lost, normal needle retention being 4 to 7 complements (fig. 1). In extreme cases only the current-year needles were left. Distinctively, the height growth of pine was in some cases extremely retarded as early as the summer of needle loss. In shortened, peculiar-looking 1987 shoots, needles also were shortened, reaching only half of their normal length.

The exceptional winter and, especially, the following 1987 growing season revealed many other phenomena (3) which could be linked together and which indicated that something had happened to the root systems. Roots have proven to be very susceptible to frosts (4) in comparison to above-ground tree parts (6).

The climate of winter 1986/1987 was exceptional. Especially in the above-mentioned needle loss area, the snow cover in December 1986-January 1987 was negligible or nil; e.g., 20-30 cm shallower than the long-term average (5). At the same time the air temperature decreased quickly to as low as -40 C. The cold period lasted 1.5 months. A combination of snowlessness and heavy frost is very rare; the former alone happens two to three times in a century and together with the latter even more rarely (1).

Root Stress Experiments

It was postulated that the root injuries of Scots pine were caused by exceptional climatological conditions. A pilot study with root stress was conducted in autumn 1987, soon after the appearance of the needle loss. It was done on a dry, sandy pine heath at Hietaperankangas in the Kivalo research area, 50 km east of Rovaniemi. The area had suffered from the exceptional conditions of the previous winter which had reduced average needle retention to three complements.

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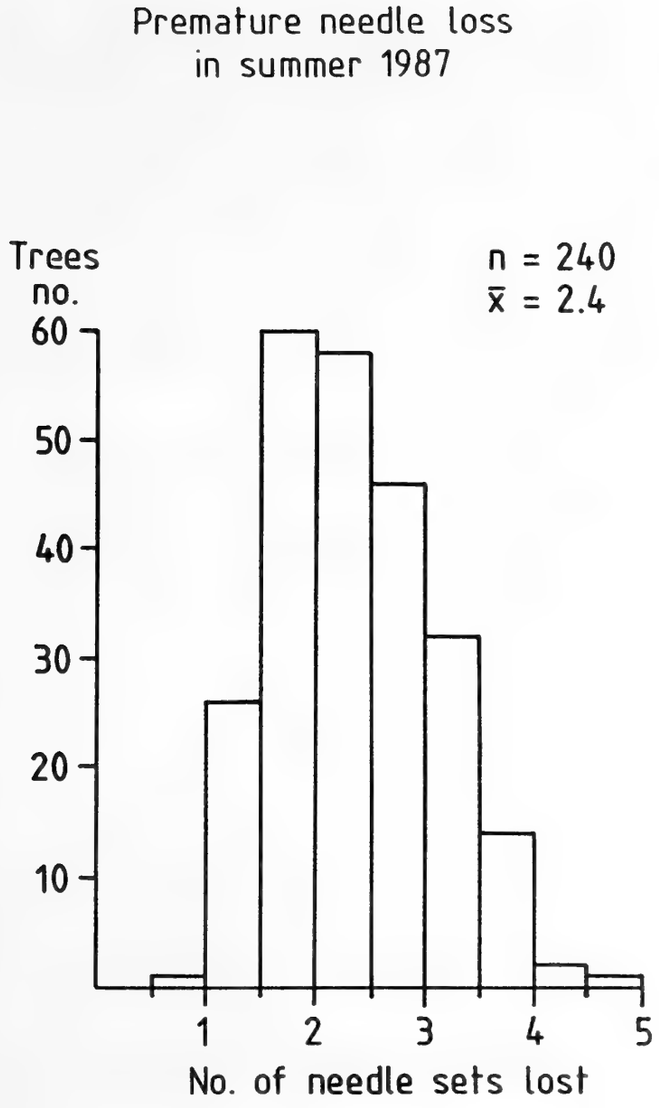

Figure 1.—Number of oldest needle complements of Scots pine lost prematurely in northern Finland in July 1987. Normal long-term needle shedding should be 1.0 needle complement annually.

In two replicates, roots were treated in two different ways. With the use of 10 x 10 m polyethene houses with open walls, the soil surface was kept free from snow in mid-winter 1987/1988 to expose roots to natural frost. In the other treatment, the soil surface was sprayed with water so that an ice layer mixed with the snow formed on the soil surface. The aim of both treatments was to imitate the climatical conditions of the previous winter. Control plots of the same size consisted of untreated trees. The experimental stand consisted of natural reproduction 35 years old and from 2 to 5 m tall. Snow and ground frost depth and air and soil temperatures were measured. At the end of February 1988, when air temperature at 2 m above ground was -26 C, soil temperature at 0.20 m depth was -16 C.

Artificial Needle Loss

At the beginning of July 1988, precisely a year after the previous year's needle loss, the oldest needles started to turn yellow in plots which either had been snowless or had an ice layer in mid-winter. In one month's time, needles turned brown and shed without any signs of defoliating fungal pathogens.

Needle loss in treated trees ranged from 17 to 24% (0.5-0.7 needle complements) when calculated in the spring based on the over-wintered needle complements (table 1). After the formation of the 1988 needle complement, total needle retention was greater in the control trees than in treated plots. There also was a significant difference in the mortality of pines in treated plots (7.8-11.7%) in comparison to untreated plots (0%).

The height increment in 1986, the year before the severe winter and needle loss, was considered normal. Thus, the 1986 height increment was given a value of 100%. The 1987 height increments of all trees were 54-62% of the 1986 increment, due to the effects of the winter of 1986/1987. The 1988 height increments of the control trees declined even further, to 35% of the 1986 level. However, in artificially stressed trees, the 1988 height increments were even less, 23-28% of the 1986 level. Dead saplings were not included in these analyses.

Table 1.—Effect of artificial root cold stress on the needle retention and mortality of Scots pine in the dry pine heath at Hietaperankangas.

	Needle retention				
	Winter 1987/1988	Yellowing in July 1988		Autumn 1988	Tree Mortality
Treatment	No. of Complements	No. of Complements	%	No. of Complements	%
Snowless	2.9	0.5	17.2	3.4	7.8
Ice cover	2.9	0.7	24.1	3.2	11.7
Control	2.8	0.1	3.6	3.7	0.0

Role of Root Cold in Needle Loss

The premature yellowing of oldest Scots pine needles was duplicated artificially by imitating root stress due to the snowless but cold winter of 1986/1987. There is no doubt that by sufficiently stressing the root system enough with frost cold, the oldest needles will turn yellow and die soon after the growing season has started, and much earlier than oldest needles normally turn yellow at the end of the season. A similar yellowing is seen in trees which have been moved and replanted in early summer, and during which part of the roots were cut. Experimental results of 1988 were clear and similar to findings in 1987, though needle yellowing certainly has been affected by both the natural and artificial root stress.

As is well known, needles and shoots of conifers are resistant to heavy frosts in mid-winter, in boreal zones at least to -70 C (6). However, roots are not as hardy and do not harden as quickly in the autumn and winter as above-ground parts. Their function can be disturbed even at temperatures as high as -10 C and severe injuries occur at -20 C (4). Thus, in the experiment during the relatively mild winter of 1987/1988 but especially in the cold, snowless winter of 1986/1987, roots were not hardy enough, and they were damaged during the rapid decrease of temperature in early December or later in the month (fig. 2). With injured roots, it does not matter whether or not the sandy soil surface thaws rapidly in spring; injured roots cannot take up water and minerals effectively enough.

It can be concluded from the above that trees regulated their water and nutrient balance successfully by replacing, in part, the normal transpiration stream from roots with movement of nutrients (and water) from older parts to the new growth. Older needles had lowered levels of

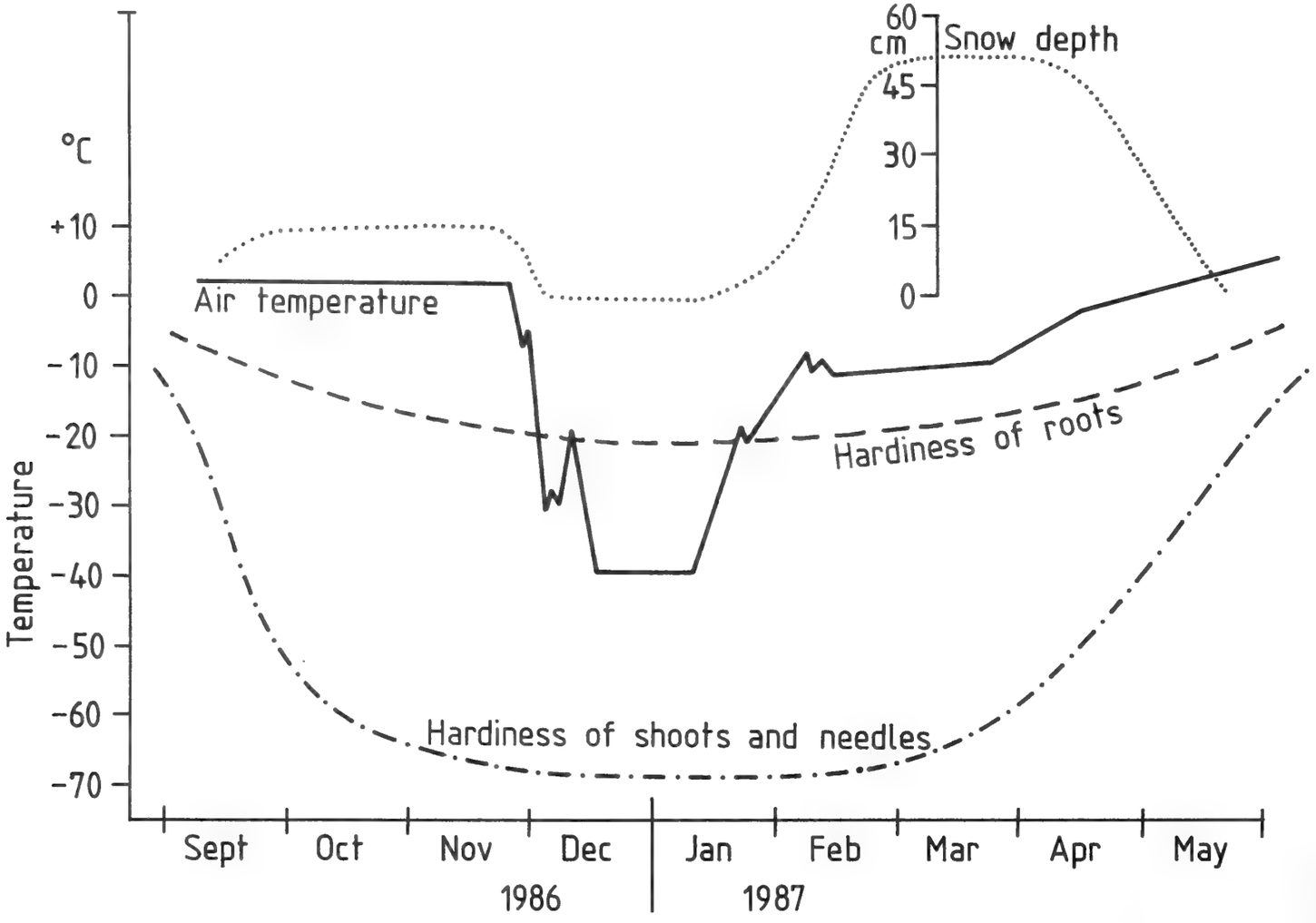

Figure 2.—Schematic presentation of the major events which led to the sudden mid-summer loss of oldest Scots pine needles in 1987 in northern Finland. At the end of the wet and snowy autumn of 1986, the snow melted. A rapid drop in temperature in December froze the surface water on the soil surface and, due to very cold weather, the temperature of the root zone in the soil dropped sharply in stands without any protection against the cold. Needles and shoots were hardy enough and no direct visible cold damage could be seen in them, but root systems could not withstand the frost and were injured. This led secondarily to disturbances in nutrient and water balance, which, in turn, led to reabsorption of nutrients from older needles and, finally, to needle yellowing.

certain mobile nutrients soon after needle loss (7). Thus, the premature yellowing seems to have caused, secondarily, mineral reabsorption from the oldest needles. This conclusion is supported by Fink's finding (2) that initial needle injuries started in the vascular bundle rather than in the mesophyll. He also concluded that root injuries caused by severe climate were the primary reason for needle yellowing.

The Future of the Affected Trees

In pine stands surrounding the experimental site, trees which suffered the root stress of 1986/1987 had started to recover as early as 1988. This was best seen by comparing needle lengths of the years 1986-1988; needles of the 1986 and 1988 complements were of normal length, but the 1987 complement was only half of normal length. In the summer of 1988, old needles did not shed. Thus, needle retention increased by one complement. This indicates that the reasons for the 1987 needle loss were acute and short-lived. New vigorous root production replacing dead roots, especially near the soil surface, was observed in the summer of 1988. It is assumed that this replacement will take years, which in turn will lower both radial and height increments for possibly as many as 10 years. Normally, pine heaths have abundant mushrooms, fruiting bodies of fungi which form mycorrhiza with pines. In the summer of 1987 not a single mycorrhizal fruiting body was formed in stands that had experienced needle loss. In 1988 the mushroom yield was reasonable again. The beginning of the growing season 1989 has indicated that the recovery is continuing.

Literature Cited

1. Anon. 1987. Finnish climate. Monthly review. Finnish Meteorological Institute.

2. Fink, S. 1989. Microscopical aspects of prematurely yellowing needles in Lappish trees. Abstract of Papers, Annual Forest Research Seminar. Finnish Forest Research Institute, Rovaniemi Research Station, 1 p.

3. Jalkanen, R. 1988. Karisevatko viimeisetkin neulaset Pohjois-Suomen puista? [Will there be any needles left on Scots pine in northern Finland?]. Teollisuuden metsaviesti 1/1988:8-11.

4. Lindstrom, A., Nystrom, C. 1987. Seasonal variation in root hardiness of container-growth Scots pine, Norway spruce, and lodgepole pine seedlings. Can. J. For. Res. 17:787-793.

5. Ritari, A. 1989. Snow and soil frost conditions in northern Finland during winter 1986-1987. Nord (in press).

6. Sakai, A., Weiser, C. J. 1973. Freezing resistance of trees in North America with reference to tree regions. Ecology 54:118-126.

7. Tikkanen, E. 1989. [Climate and air pollution - reasons for the needle loss in the summer of 1987]. Abstract of Papers, Annual Forest Research Seminar. Finnish Forest Research Institute, Rovaniemi Research Station, 3 p.

Current Season Needle Necrosis: A Needle Disorder of Unknown Etiology on Noble and Grand Fir Christmas Trees in the Pacific Northwest[1,2]

Gary A. Chastagner, John M. Staley, and Kathy L. Riley[3]

Abstract.—One of the major disease problems encountered in Noble as well as Grand fir Christmas tree plantations is a needle disorder of unknown etiology referred to as "current season needle necrosis." Current season needle necrosis was observed on Noble fir trees in 98 and 96% of the 52 plantings examined during May and October 1984, respectively. The percentage of affected 1983 and 1984 needles was 12 and 23%, respectively. Initial symptoms of current season needle necrosis appeared on the current season needles during early June in 1985 and 1986. Initially, symptoms consisted of tan discolored bands which expanded and turned reddish-brown by summer. Branches in the upper portion of Noble fir trees had twice as many symptomatic needles compared to branches in the middle and bottom of the trees. Branch position had no effect on the incidence of symptomatic needles on Grand fir trees. No evidence of a pathogen was detected in symptomatic needles during late June. Shading trees or applications of calcium chloride during shoot elongation significantly reduced the incidence of symptomatic needles on Noble fir.

Introduction

Approximately 20% of the total Christmas trees produced in the United States come from western Washington and Oregon. In 1986, there were 31,475 ha of trees grown in plantations and 13,480 ha in natural stands. The farm gate value of trees was $104 million in 1988. During the early 1980's, approximately three times as many trees were being planted as were harvested (11). Although Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) is the most common tree being harvested, its share of the total production has been decreasing, while the percentage of true firs, principally Noble fir (*Abies procera* Rehd.) has been increasing (6,11,13), The increase in true fir production has occurred because of concern about a softening of the Douglas-fir market and the superior postharvest keeping qualities of Noble fir compared to Douglas-fir Christmas trees (10, Chastagner, unpublished).

The natural range of Noble firs occurs on the west side of the Cascade Mountains between 427 and 1,830 m elevation from the United States-Canadian border in the north to the northern portion of California in the south (12). Small patches of Noble fir also occur in the coastal range in southwestern Washington and northwestern Oregon.

The expansion of Noble fir Christmas tree plantings in western Washington and Oregon has meant an increase in concern about disease problems. One of the major disease problems encountered in Noble as well as Grand fir (*Abies grandis* (Dougl.) Lindl.) Christmas tree plantations is a needle disorder of unknown etiology being referred to as "current season needle necrosis" (5). In 1984, research was initiated to determine the prevalence and distribution of this needle disorder on Noble fir Christmas trees, characterize symptom development on Noble and Grand fir Christmas trees, and examine factors which affect the severity of symptom development.

Materials and Methods

Prevalence and distribution of current season needle necrosis.—During May and October, 1984, 52 plantings of Noble fir Christmas trees in western Washington and Oregon were surveyed to determine the prevalence and distribution of current season needle necrosis. Plantings were located as far north as Blaine, Washington and as far south as Salem, Oregon. Fifteen systematically selected trees, 1.5 to 2.5 m in height, were tagged and

[1] Paper presented at the I.U.F.R.O. W.P. S2.06.04 Foliage Disease Conference, 29 May - 2 June 1989, Carlisle, Pennsylvania.

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examined for the presence of current season needle necrosis in each planting. The presence of symptoms on 1983 needles was determined when trees were examined in May prior to bud break while the presence of symptoms on the 1984 needles was determined in October.

Development of current season needle necrosis symptoms.—The development of symptoms on Noble and Grand fir Christmas trees was examined by establishing plots in commercial Grand fir and Noble fir Christmas tree plantations located near Puyallup, Washington during 1986. Each plot consisted of ten 2-2.5 m tall trees, which had symptoms of current season needle necrosis on their 1985 needles. Prior to bud break, a single branch in each quadrant of the top, middle and bottom portion of the trees was tagged. The development of current season needle necrosis symptoms was assessed periodically during the spring and summer by examining each tagged branch and recording the number of symptomatic needles, healthy needles and needle scars. Shoot length measurements were made at each examination time.

Data were also collected on the development of symptoms on smaller 1-1.5 m tall Noble fir and Grand fir in a plantation near Puyallup during 1986. Four hundred eighty Grand fir (Clearwater, Idaho seed source), 230 Noble fir and 250 Noble fir from 053 and 440 seed sources, respectively, were tagged prior to bud break. The number of trees on which symptoms developed was recorded periodically during spring and summer.

Isolation and examination of symptomatic needles.—Noble fir and Grand fir needles with symptoms of current season needle necrosis were collected on 26 June 1985 for histological examination and isolations. Symptomatic needles were sectioned using a freezing microtome and examined at 400X with a compound microscope. Isolations were also done from ten needles with symptoms from each of ten trees. Needles were surface sterilized for 1 minute in 0.5% NaOCl, blotted dry, and 1-mm-thick sections were then plated onto potato-dextrose agar (PDA, Difco) and incubated at 20C. Plates were examined weekly for approximately 4 weeks for growth from individual pieces of needles.

Examination of factors affecting symptom development.—During 1985 and 1986, experiments were established to examine what affect a number of factors might have on the development of current season needle necrosis on Noble fir Christmas trees. During 1985, trees with a previous history of current season needle necrosis in a commercial plantation near Puyallup, WA were tagged and the extent of symptoms on the 1984 needles was rated. Individual trees with uniform levels of symptomatic 1984 needles in each of 16 blocks were assigned to each treatment and non-treated trees served as a check. Treated trees were separated from each other by one or more nontreated trees. Treatments consisted of covering trees prior to bud break with 53% shade cloth on 13 May or maintaining high soil moisture during shoot elongation by flooding 3-m-diameter basins beneath each tree with water on 24 May, 17 and 25 June, 7, 19, 24, and 29 July. Additional treatments consisted of spraying developing shoots and needles with the antitranspirant Folicote (a hydrocarbon wax emulsion, Crystal Soap and Chemical Co., Lansdale, PA) diluted 1:19 in water, or Bravo 500 (chlorothalonil) at 1.32 g a. i./liter. Applications of these materials were made on 24 May, and 3, 17 and 27 June using a Solo backpack sprayer equipped with an 8003LP nozzle at 1.05 kg/cm^2. Single 1985 branches in the upper third of each tree were collected on 9 August and the incidence of current season needle necrosis was determined by counting the number of symptomatic needles, needle scars and healthy needles on each branch.

Foliar applications of calcium chloride were applied to Noble fir trees during 1985 and 1986. In 1985, calcium chloride at 0, 1.0, 2.5 and 5.0 mg/ml plus Ortho X-77 at 1.3 ml/l were applied to individual 1.5 to 2.0 m tall trees in a commercial plantation near Puyallup, WA. Four applications were made at 10-day intervals starting on 23 May. The plot design was a randomized complete block with a single tree per treatment in each of 16 blocks. In 1986, two plots were established to determine the effect of foliar applications of calcium chloride on the development of current season needle necrosis. Applications of calcium chloride at 0, 1.0, 5.0 and 10.0 mg/ml plus Ortho X-77 at 1.3 ml/l were applied to individual trees in both plots on 5, 18 and 28 May. One additional application was applied to the trees in plot 2 on 7 June. Plot designs were randomized complete blocks with a single tree per treatment in each of 20 and 15 blocks in plots 1 and 2, respectively.

All 1985 and 1986 calcium chloride treatments were applied with a Solo backpack sprayed equipped with a 8003LP nozzle at 1.05 kg/cm^2. Shoot length measurements were taken on a single shoot in the upper portion of each tree at each application time. The percentage of needles with current season needle necrosis was determined during late July and August. Phytotoxicity associated with the applications was also determined by rating the incidence of affected needles on a scale of 0-10, where 0 = no injury and 10 = 91-100% of the needles with injury.

Results

Prevalence and distribution.—Current season needle necrosis was observed on trees in 98 and 96% of the plantings examined during May and October, respectively. In May, 12% of the trees examined had symptoms on their 1983 needles, while 23% had symptoms on the 1984 needles examined during October.

Symptom development.—Initial symptoms consisted of tan discolored bands on the needles. In some cases the area of discoloration expanded and involved the distal

portion of the needle or the entire needle. These affected portions of the needles turned a reddish-brown by early summer and needles with extensive symptom development were generally shed, particularly on Grand fir.

Initial symptoms occurred on both the Noble and Grand fir on 19 June 1986. The incidence of current season needle necrosis increased rapidly during June and July. There was no statistical difference in the incidence of symptoms which developed on needles in the different quadrants of the tree (data not shown). Branches in the upper portion of the Noble fir trees had twice as many symptomatic needles compared to branches in the middle and bottom of the trees (fig. 1). The Grand fir had approximately twice as many symptomatic needles as the Noble fir and branch position had little effect on symptom development (fig. 2).

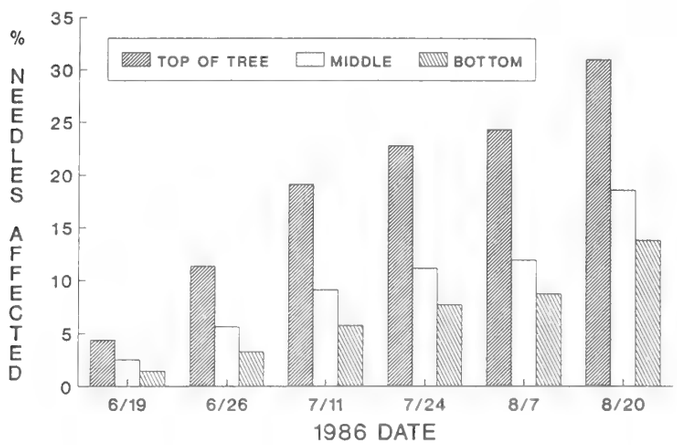

Figure 1.—The development of current season needle necrosis on branches in the top, middle and bottom portions of 10 Noble fir Christmas trees.

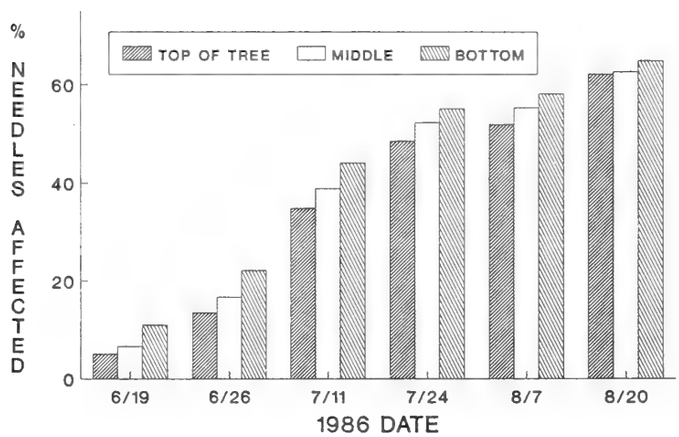

Figure 2.—The development of current season needle necrosis on branches in the top, middle and bottom portions of 10 Grand fir Christmas trees.

In the plantings of smaller Grand fir and noble fir examined in 1986, initial current season needle necrosis symptoms were observed on 9 June and symptom development occurred most rapidly during the next 4-week period. The incidence of trees with current season needle necrosis was slightly higher for the Grand fir and there were no differneces between the two Noble fir seed sources in the percentage of trees with symptomatic needles (fig. 3).

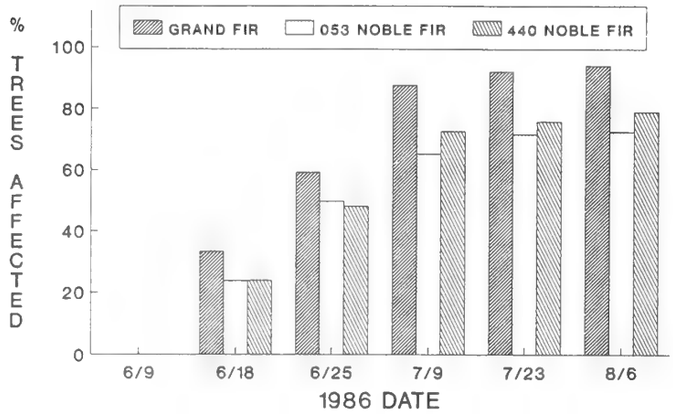

Figure 3.—Development of current season needle necrosis on 480 Grand fir and 230 and 250 Noble fir from 053 and 440 seed sources, respectively.

Isolation and examination of needles.—No evidence of a pathogen was detected upon examination of the sections of symptomatic needles. Except for one piece of needle, no growth developed on the 100 pieces of Noble and Grand fir needles plated onto the PDA.

Factors affecting symptom development.—Slightly more than 20% of the needles on the nontreated check trees had symptoms of current season needle necrosis on 9 August. Applications of Folicote, Bravo 500 and basal irrigation during shoot elongation had no effect on the incidence of symptomatic needles, while covering trees with shade cloth during shoot elongation significantly reduced the incidence of symptomatic needles (table 1).

Applications of calcium chloride significantly reduced the incidence of symptomatic needles and there was a negative correlation between the incidence of symptomatic needles and the concentration of calcium chloride applied during shoot elongation (fig. 4). In 1985, shoot lengths increased from 3.4 to 20.3 cm during the period applications were made. In 1986, shoot lengths increased from 1.1 to 9.2 cm and 1.7 to 11.4 cm in plots 1 and 2, respectively, during the period applications were made. Although no phytotoxicity was observed on the trees treated with calcium chloride during 1985, reddish discolored needle tips were present on the trees in both plots by 7 June during the 1986 tests (table 2).

Table 1.—Effect of various treatments during shoot elongation on the incidence of current season needle necrosis on Noble fir Christmas trees

Treatments[1]	Percent symptomatic needles[2]
Check	20.2 a
Basal irrigation	24.3 a
Antitranspirant (Folicote)	15.9 a
Fungicide (Bravo 500)	18.4 a
53% shade cloth	3.1 b

[1] Treatments were applied to individual trees in each of 16 blocks. The soil beneath the trees receiving the irrigation treatments was flooded on 24 May, 17 and 25 June, 7, 19, 24 and 29 July. Folicote (diluted 1:19 in water) and Bravo 500 at 1.32 g a.i./l were applied to trees on 24 May, 3, 17 and 27 June and the shade cloth was placed over the trees on 23 May 1985.

[2] Data collected on 9 August 1985. Numbers followed by the same letter are not significantly different, $P < 0.05$, Duncan's mulitple range test.

Discussion

Although only a limited number of diseases and disorders have been reported on Noble fir (2,3,7,8), surveys of Noble fir Christmas tree plantations in western Washington and Oregon indicate that current season needle necrosis is prevalent throughout the production areas examined. Approximately twice as many needles on the survey trees exhibited symptoms of this needle disorder in 1984 compared to 1983, indicating that there can be considerable variation in disease incidence from one year to another. Outside of the Pacific Northwest, this disorder has been observed on Noble fir growing near

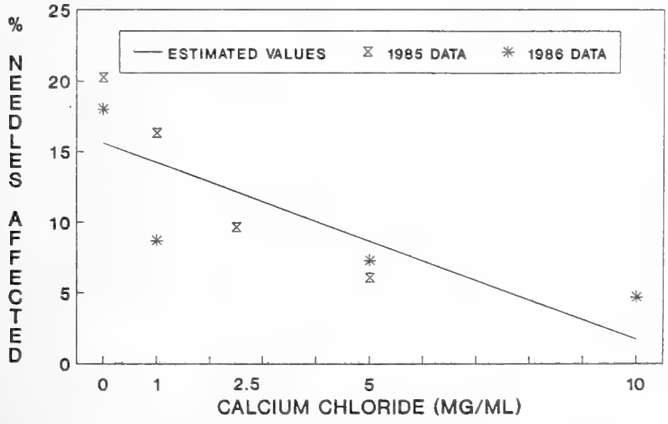

Figure 4.—Relationship between increasing concentrations of calcium chloride and the percentage of Noble fir needles with current season needle necrosis during 1985 and 1986 tests ($Y^1 = 15.6 - 1.4X$, $r = 0.807$, $P<0.05$).

Table 2.—Phytotoxicity associated with applications of calcium chloride to Noble fir Christmas trees during 1985 and 1986

Concentratin[1] mg/ml	Percentage of needles with tipburn		
	1985	1986	
		Plot 1	Plot 2
0.0	0.0	0.0	0.0
1.0	0.0	0.0	0.7
2.5	0.0	-	-
5.0	0.0	8.5	4.7
10.0	-	24.5	36.0

[1] Applications of calcium chloride plus 1.25 ml Ortho X-77 per liter were applied to 16 trees in 1985 and 20 and 15 trees in Plots 1 and 2, respectively during 1986. Data were collected on 9 August 1985 and 17 June (Plot 1) and 31 July (Plot 2) in 1986.

Newbury Berks in the United Kingdom (J. H. Godwin, personal communication). In addition to Noble fir, this disorder has commonly been observed on Grand fir being grown as Christmas trees in Oregon, Washington, Idaho and the lower Fraser River Valley in southwestern British Columbia, Canada.

Initial symptoms of current season needle necrosis occurred during shoot elongation as tan discolored bands on the newly developing needles. Later, these bands turned a reddish-brown. The lack of signs of a pathogen and growth from surface-sterilized symptomatic needle tissue shortly after symptom development indicates that current season needle necrosis is probably not caused by a parasitic pathogen. If symptomatic needles are examined several months after initial symptoms appear, fruiting bodies of a number of fungi can be found on the dead portions of the needles. Isolations from needles at this time yield a variety of fungi which are presumed to be saprophytes that have colonized the necrotic tissue.

At this time, the cause of current season needle necrosis in unknown. In the United Kingdom, there is speculation that the use of certain types of herbicides results in the development of current season needle necrosis (J. H. Godwin, personal communication). However, we have observed this disorder on trees in plantations and container-grown stock in the Pacific Northwest where herbicides have not been used. Air pollutants, such as sulfur dioxide, ozone and hydrogen flouride, are known to injure conifer needles (9). Since sulfur dioxide and ozone injury are more prominent on middle-aged and older needles, it is unlikely they are the cause of current season needle necrosis. Although current-year needles are the most sensitive to hydrogen flouride, it is unlikely that current season needle necrosis is caused by this pollutant because Grand fir is less susceptible to hydrogen flouride than Douglas-fir, and current season needle necrosis symptoms are rarely seen on Douglas-fir in the Pacific Northwest.

Our tests indicate that shading or applications of calcium chloride during shoot elongation are effective in reducing the incidence of this needle disorder on Noble fir Christmas trees. Although Noble fir are considered intolerant of shade (1), some are grown in partial shade in the United Kingdom, and these trees are much less likely to be affected by this disorder than trees grown in exposed areas (J. H. Godwin, personal communication). The effectiveness of foliar applications of calcium chloride in reducing the incidence of this disorder suggest that calcium deficiencies during shoot elongation may be associated with the development of current season needle necrosis. In addition to reducing factors such as light levels, temperature and evaporation, shading trees during shoot elongation reduced shoot growth by 21% (data not shown). Because of the limited mobility of calcium within rapidly growing plant tissues, this reduction in shoot growth may affect the availability of calcium in the developing needle tissues. Because of the potential for phytotoxicity, the use of foliar applications of calcium chloride to reduce the incidence of current season needle necrosis on Noble and Grand fir Christmas trees appears to be limited.

Additional research is needed to determine the basis for the reduction in the incidence of current season needle necrosis associated with the use of shading and/or foliar applications of calcium chloride. In addition, information on the susceptibility of Noble and Grand fir provenances to this disorder and the affect of cultural practices, such as fertilization and liming, on the incidence of current season needle necrosis is needed so that a practical means of controlling this problem can be obtained.

Acknowledgements

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Literature Cited

1. Baker, F. S. 1949. A revised tolerance table. J. For. 47:179-181.
2. Bega, R. V. 1978. Diseases of Pacific Coast Conifers. USDA For. Serv. Agric. Handb. 521. 206 p.
3. Boyce, J. S. 1948. Forest Pathology. 2nd ed. McGraw-Hill, N.Y. 550 p.
4. British Forestry Commission. 1957. Exotic forest trees in Great Britain. Bulletin 30, 167 p.
5. Chastagner, G. A., Staley, J. M. 1985. Disorders and diseases observed during a survey of Noble fir Christmas trees in western Washington and Oregon. (Abstr.) p. 108 in Proc. 33rd Annual Western Int'l. For. Dis. Work Conf., Olympia, WA. Sept. 24-27. p. 108.
6. Douglass, B. S. 1983. Report on the 1983 marketing season. Northwest Lookout 16(2):2-6.
7. Franklin, J. F. 1962. Noble fir - A bibliography with abstracts. USDA For. Serv. PNW For. Range Exp. Sta. Res. Paper 46. 41 p.
8. Hepting, G. H. 1971. Diseases of Forest and Shade Trees of the United States. USDA For. Serv. Agric. Handb. 386. 658 p.
9. Miller, P. R. 1978. Abiotic diseases. p. 5-41. In R. B. Bega (ed.). Diseases of Pacific Coast Conifers. USDA For. Serv. Agric. Handb. 521. 206 p.
10. Nanny, B. 1986. Noble fir: That mysterious difficult and beautiful Christmas tree. Northwest Lookout 19(3):10-12.
11. Proebsting, W. M. 1983. Harvest and planting rates of Christmas trees in Oregon. Northwest Lookout 16 (2):20-22.
12. Staebler, G. R. 1958. Silvical characteristics of Noble fir. USDA For. Serv. PNW For. Range Exp. Sta. Silvical Ser. 5, 12 p.
13. U.S. Forest Service. 1960. Production and marketing of Christmas trees in the Pacific Northwest in 1959. Region 6, 21 p.

Epidemiology and Control of *Ploioderma Lethale* on *Pinus Nigra*[1]

John Longenecker[2] and Barry Towers[3]

Abstract.—Chlorothalonil was more effective than Maneb + zinc in controlling *Ploioderma lethale* needlecast of 18-year-old *Pinus nigra* in a field test on 25 trees. Spore discharge was correlated with rainfall. A tentative spray schedule based on needle elongation is proposed.

Ploioderma lethale (Deam.) Darker has been reported as causing a needlecast of hard pines in Pennsylvania since at least 1951 (1). The Pennsylvania Departments of Agriculture and Environmental Resources records maintained by the authors show that this disease has been occasionally observed on Austrian pine (*Pinus nigra* Arnold) and pitch pine (*Pinus rigida* Mill.) in more recent years. In 1976 severe browning and needlecasting of *P. nigra* were observed at the Chester Water Authority in Lancaster and Chester Counties. The condition had been present for at least two years. Approximately 35% of the trees on 61 hectares of 18-year-old *P. nigra* were infected by *P. lethale*; the remaining 65% appeared to have resistance to the fungus since infected and healthy trees were randomly interspersed. Because of the existing threat to the watershed, a study was initiated to determine the epidemiology and control of *P. lethale* on *P. nigra*.

Materials and Methods

The experimental plots were located on a 32,300 hectare watershed in southeastern Pennsylvania (39° 44' N, 76° 04' W, elevation 90 m). Temperature, RH and rainfall were monitored 9 May through 20 August 1978, utilizing a hygrothermograph (Bendix Corporation) and a tipping rain gauge (Weather Measure Instrument Corporation). Spores were trapped from 22 May through 20 August utilizing a Rotorod spore sampler. Needle and candle elongation were measured daily on 10 branches from 22 May through 20 August to relate tree development to spore release.

Twenty-five heavily infected trees were randomly selected to be treated with one of four different spray regimes, Maneb + zinc (Dithane M-45, 3.9 ml formulated product/liter) or chlorothalonil (Bravo 6F, 7.8 ml formulated product/liter) applied three and five times, respectively, plus a control. A spreader/sticker was added to all sprays. Sprays were applied with a backpack mistblower at 14-day intervals starting on 23 May 1978.

On 4 May 1979 one branch at 1.3 m height on the north and south sides of each of the 25 trees was selected to evaluate spray effectiveness. Ten needles were excised from each twig in a descending helical manner, evaluated independently by the authors, and rated for needlecast presence.

Results

Ploioderma lethale spores were first trapped on 7 June. Discharge was closely correlated to periodicity of rainfall (fig. 1). There was no apparent correlation of spore discharge with relative humidity. First spore discharge was noted when needle length and candle elongation were approximately 35 and 65% of total, respectively (fig. 2). Of the two fungicides applied, chlorothalonil gave better control at both the three- and five-spray applications (table 1).

Discussion

Based on the results, chlorothalonil (Bravo 6F) gave satisfactory control of *P. lethale* needlecast of *P. nigra* when applied under the conditions of this study. Three applications appeared to be adequate to control the disease.

Although the application of Maneb + zinc reduced disease development compared to that on unsprayed controls, Maneb + zinc was less effective than chlorothalonil.

[1] Paper presented at the I.U.F.R.O. W.P. S2.06.04 Foliage Disease Conference, 29 May-June, 1989, Carlisle, Pennsylvania.

[2] Chief, Division of Agronomic Services, Pennsylvania Department of Agriculture, Harrisburg, PA 17110

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This study suggests that a spray schedule based upon three applications at two-week intervals beginning when the needles have reached 30% of their expected elongation will give satisfactory disease control. It is recognized that the comparison of needle growth and candle elongation to spore discharge represents one year's data and should be confirmed by subsequent investigations.

Acknowledgement

The cooperation of Mr. Herman Latham of the Chester Water Authority in obtaining field data is gratefully acknowledged.

Literature Cited

1. Morris, C. L. 1953. Chemical control of *Hypoderma lethale* on pitch pine. Plant Dis. Rep. 37: 368-370.

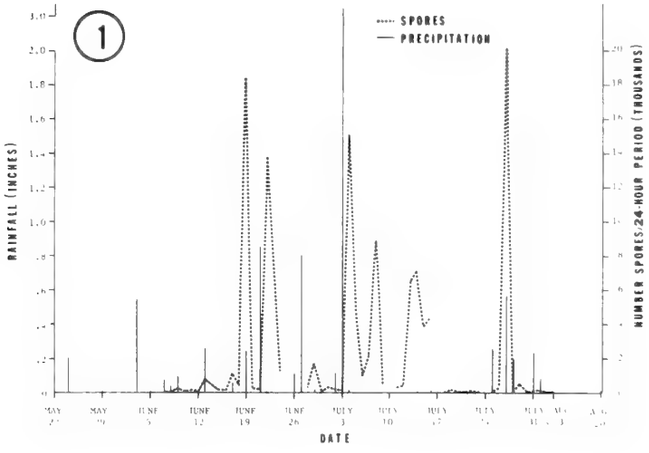

Figure 1.—Relation of *Ploioderma lethale* spore release to rainfall, Chester County Pennsylvania, 1978.

Table 1.—Effectiveness of Maneb + zinc and chlorothanlonil in controlling *Ploioderma lethale* needlecast of *Pinus nigra*

Spray Schedule	Number of Needles with *P. lethale* Infection (20 needles/tree on each of 25 trees)		
	Observer 1	Observer 2	Average
None (Control)	20.0	20.0	20.0
Maneb + Zinc (3X)	12.4	10.8	11.6
Maneb + Zinc (5X)	15.0	15.4	15.2
Chlorothalonil (3X)	0.4	0.6	0.5
Chlorothalonil(5X)	0.0	0.0	0.0

X = applications

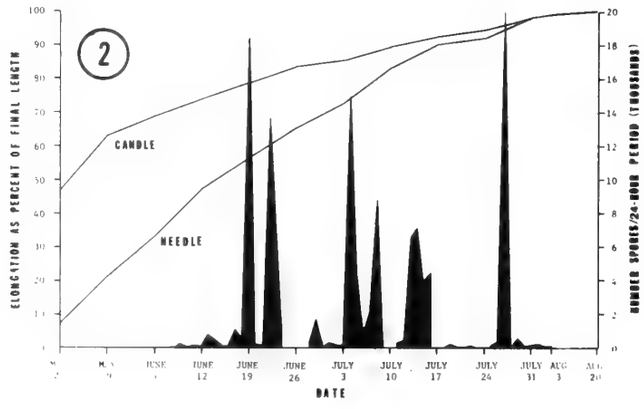

Figure 2.—Relation of *Ploioderma lethale* spore release to *Pinus nigra* needle and candle elongation, Chester County, Pennsylvania, 1978.

Seasonal Development of Symptoms and Fruiting Bodies of *Dothistroma Septospora* on *Pinus Thunbergii* in Shimane Prefecture, Japan[1]

Yasuo Suto[2]

Abstract.—Some ecological characteristics of needle blight of *Pinus thunbergii* caused by *Dothistroma septospora* were studied in Shimane Prefecture, Japan. Symptoms appeared from October and stromata began to develop in December. Conidia were produced from April to August of the following year; the peak production was in June. Conidia were produced at temperatures from 15 to 25 C, with an optimum of 20-25 C. These coincided with average temperatures of the whole period of conidial production, and of the period of the highest production in the field, respectively.

Introduction

Dothistroma needle blight, caused by *Dothistroma septospora* (Doroguin) Morelet, is one of the serious needle diseases of *Pinus thunbergii* Parl. planted in gardens and parks in Shimane Prefecture, Japan (7, 8). The reddish-brown lesions on the infected needles, and premature defoliation prevent normal growth of the trees and destroy their esthetic value. In order to control a disease, it is necessary to know the ecological characteristics of the disease. This paper deals with the results of experiments on seasonal development of symptoms and fruiting bodies of the fungus, and influence of temperature on the latter.

Materials and Methods

Field experiments.— The experiments were conducted on four 30- to 50-year-old *P. thunbergii* in the Matsue Castle Park, Matsue City, Shimane Prefecture, Japan. Needles of these trees had been heavily infected with *D. septospora* annually. Development of symptoms and signs was observed from October 1982 to October 1984, and infected needles were collected at about 2-week intervals. Twenty stromata were sectioned by hand, and stromatal development and conidial production were examined microscopically.

Infected needles were collected at about 10-day intervals in May and June, 1985. Needles were cut into pieces with 50 stromata of the fungus, and the pieces were sprayed with 10 ml of water to release conidia from the stromata. Conidia were counted by a hemacytometer. Each trial was replicated seven times.

Laboratory experiments. — Infected needles were collected in early February 1983 and cut into 10 mm long pieces with stromata developing under the epidermis. Ten pieces were put on moistened filter paper in Petri dishes and incubated at 10, 15, 20, 25, 30, and 35 C. After 10 days, these stromata were sectioned by hand, and stromatal development and conidial production were examined microscopically. This experiment was repeated three times.

Results

Field experiments. — Symptoms appeared on many previous year needles and on some current-year needles in early October 1982 and 1983. Spots and bands were yellow, 2-5 mm in length, and resin sometimes oozed out of them. After mid-January of the following year, the lesions turned reddish brown and became prominent. On infected current-year needles, the spots and bands appeared on the distal part of needles and the needle tips beyond the lesions died. Basal parts of the needles remained green. On infected previous year needles, lesions were distributed evenly from the base to the distal end of the needles, and necrosis extended almost the entire length of the needles. Many previous year needles shed in November.

Stromata began to develop under the epidermis of the needles in mid-December as black spots, and appeared on the surface of the lesion by rupturing the epidermis from early to late January of the following year (fig. 1A, B).

[1] Paper presented at the I.U.F.R.O. W.P. S2.06.04 Foliage Disease Conference, 29 May-2 June, 1989, Carlisle, Pennsylvania.

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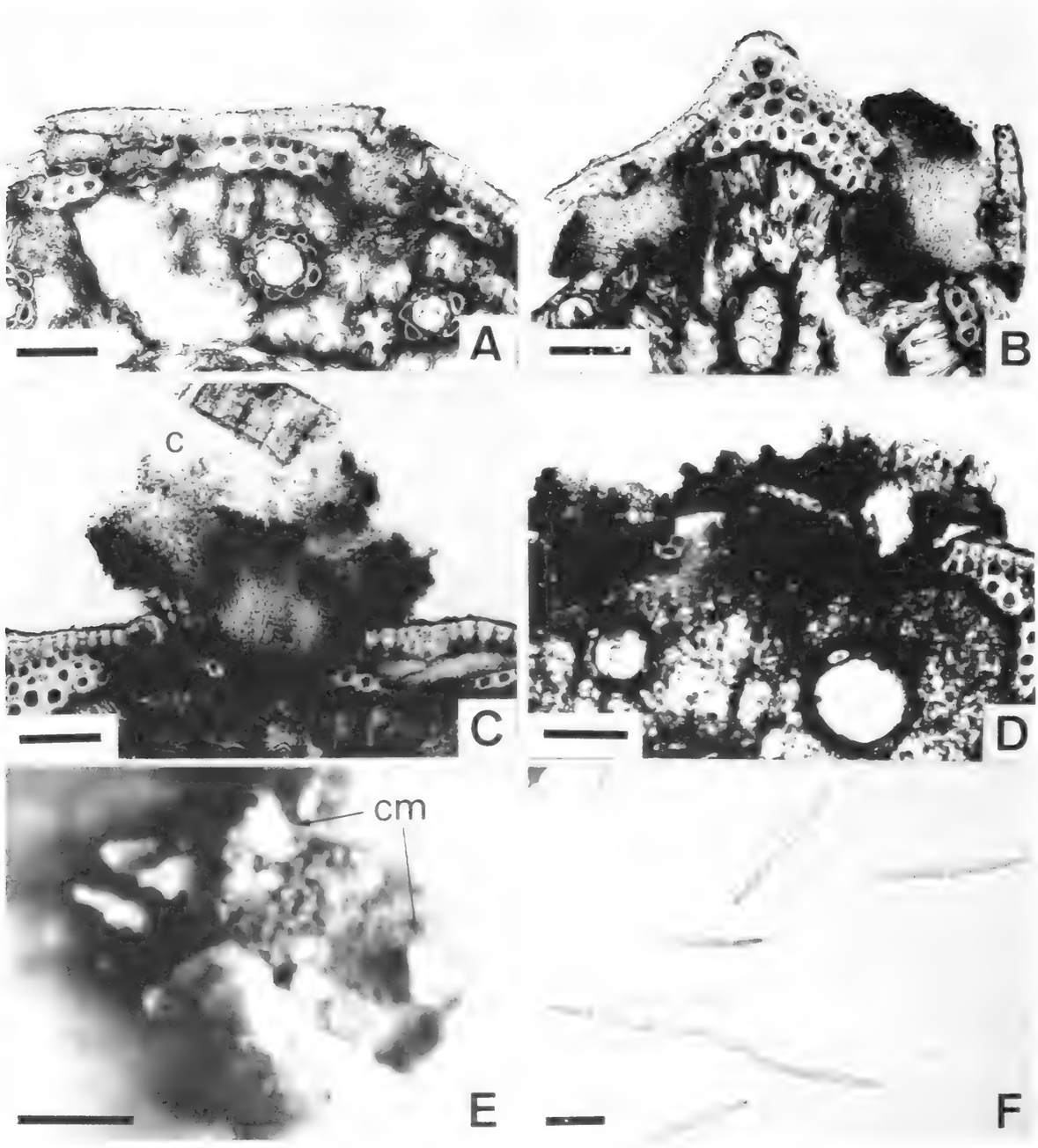

Figure 1.—Stromatal development and conidial production of *Dothistroma septospora*. A: Stromata developing under epidermis. B: Stromata appearing on the surface of the lesion by slitting epidermis. C: Stroma producing condidia (c). D: Stroma which has turned black and dried. E: Conidial masses (cm) produced on a stroma. F: Conidia. (Scales: A, B, C, D, E = 100 µm, F = 10 µm)

As shown in table 1, conidia were first detected on stromata on 2 April 1983, and on 21 April 1984. A large number of conidia was produced from late May to early July 1983, and from early June to early July 1984 (fig. 1C, E, F). Stromata then turned black and dried, and almost no conidia were detected after early August 1983, and after mid-August 1984 (fig. 1D).

In May and June, 1985, which was the peak period of conidial production, $0.7\text{-}1.8 \times 10^4$ conidia were produced per stroma.

The perfect state of the fungus, *Mycosphaerella pini* E. Rostrup apud Munk, was not found at all during the experiments.

As shown in figure 2, the 10-day average temperature was 13 and 14 C at the time of initial conidial production, 20-22 C and 22-25 C at the time of the highest production in 1983 and 1984, respectively, and 28 C at the time of final production in both years. There was rain throughout the period of conidial production, and rainfall at 10-day intervals during the period was 6-181 and 0-199 mm in

Table 1.—Seasonal conidia production of *Dothistroma septospora*

Date of collection	Stromata producing conidia	Degree of conidial production[a]
	%	
Dec. 14, 1982	0	-
25	0	-
Jan. 13, 1983	0	-
27	0	-
Feb. 19	0	-
Mar. 5	0	-
21	0	-
Apr. 2	45	+ ~ ++
23	60	+ ~ ++
May 2	25	+
20	100	++
June 4	100	++
21	100	+ ~ ++
July 4	100	+ ~ ++
18	50	+ ~ ++
Aug. 1	25	+
16	0	-
Sept. 3	0	-
19	0	-
Dec. 14, 1983	0	-
Jan. 2, 1984	0	-
18	0	-
Feb. 4	0	-
14	0	-
Mar. 5	0	-
20	0	-
Apr. 6	0	-
21	27	+
May 1	67	+
18	100	+
June 2	100	+ ~ ++
18	95	+ ~ ++
July 2	95	+ ~ ++
14	80	+ ~ ++
Aug. 1	50	+ ~ ++
18	5	+
Sept. 1	0	-
19	0	-

[a] No production, +: Light production, ++: Heavy production.

1983 and 1984, respectively. Considerable rainfall, above 100 mm at an interval, was recorded at the time of the highest conidial production in both years.

Laboratory experiments. — As shown in table 2, the epidermis of lesions was ruptured by the development of stromata at temperatures ranging from 10 to 30 C, with an optimum of 20-25 C. Conidia were produced at temperatures ranging from 15 to 25 C, with an optimum of 20-25 C.

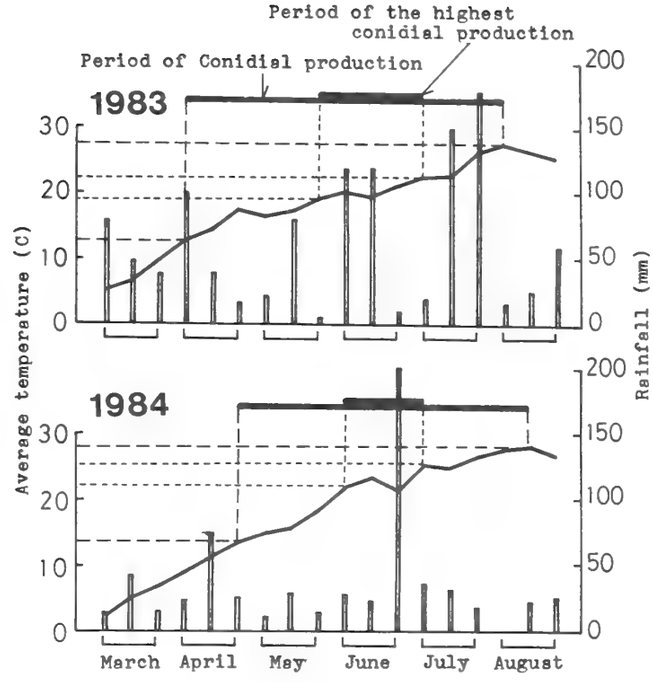

Figure 2.—Relationship between climatic conditions and production of conidia of *Dothistroma septospora*.

Discussion

Although symptoms of Dothistroma needle blight on *P. thunbergii* appeared in October, conidia of the causal fungus were produced from April to August of the following year, and peak production was in June in Shimane Prefecture. In order to control the disease, copper fungicides, which have been recognized as the most effective chemicals (4), should be applied during the period of conidial production. Control was not attempted, but it is assumed that coating needles with chemicals in June is necessary for disease control, because a large number of conidia is produced on the infected needles, the conidia are disseminated by frequent rainfall, and the average temperature of 20-23 C and moist conditions are favorable for conidial germination of the fungus (3) in that month. Furthermore, current-year needles that have to be protected from the fungus develop in June.

Brief observations on the time of conidial production by the fungus have been reported in two different regions in Japan. Conidia were produced from late May to late July with peak production in early June in Hokkaido (9), and from early March to late June with peak production from mid-April to mid-May in Tokyo (3). Time and duration of conidial production in these regions differed slightly from those in Shimane Prefecture. The perfect state of the fungus was not found in these regions nor in Shimane Prefecture. Seasonal development of the disease was reported by Funk and Parker (1) in Canada, by Peterson (4, 5) at two regions in the U.S.A., by Rack (6) in Chile, and by Gilmour (2) in New Zealand. It differed among these countries or regions in some aspects, such as presence of the perfect state, time

Table 2.—Effect of temperatures on conidial production of *Dothistroma septospora*

Temperature (°C)	Number of stromata slitting epidermis	Number of stromata producing conidia		
		Produced a little	Produced much	Total
10	10	0	0	0
15	12	3	6	9
20	22	1	17	18
25	26	1	13	14
30	2	0	0	0
35	0	0	0	0

30 stromata were used at each temperature.

of symptom appearance, and period of conidial production. The differences are assumed to be due to climatic conditions of the regions, and physiological variation of the causal fungus. Characteristics of life cycle of the fungus in Japan are summarized as follows: 1) Perfect state of the fungus is not found. 2) The disease cycle is completed in the year following the infection. 3) Conidia are not produced after summer.

The minimum, optimum, and maximum temperatures for conidial production of the fungus were 15, 20-25, and 25 C, respectively. These temperatures roughly coincided with average temperatures of the 10-day period of the initial, the highest, and the final conidial production in the field, respectively. Conidia could be easily produced in moistened Petri dishes by adjusting temperatures to the favorable range in early February when the conidia could not be produced in the field under natural conditions. These results show that conidial production on stromata is directly influenced by temperature under moist conditions, and average temperature is considered to be a good indicator of conidial production of the fungus in the field.

Literature Cited

1. Funk, A., Parker, A. K. 1966. *Scirrhia pini* n. sp., the perfect state of *Dothistroma pini* Hulbary. Can. J. Bot. 44:1171-1176.
2. Gilmour, J. W. 1981. The effect of season on infection of *Pinus radiata* by *Dothistroma pini*. Eur. J. For. Path. 11:265-269.
3. Ito, K., Zinno, Y., Suto, Y. 1975. Dothistroma needle blight in Japan. Bull. Gov. For. Exp. Sta. 272:124-140.
4. Peterson, G. W. 1967. Dothistroma needle blight of Austrian and ponderosa pines: Epidemiology and control. Phytopathology 57:437-441.
5. Peterson, G. W., Harvey, G. M. 1976 . Dispersal of *Scirrhia (Dothistroma) pini* conidia and disease development in a shore pine plantation in western Oregon. Plant Dis. Reptr. 60:761-764.
6. Rack, K. 1986 Über die jahreszeitliche Entlassung der Konidien von *Dothistroma pini* in *Pinus radiata*-kulturen des sudlichen Chile. Eur. J. For. Path. 16:6-10.
7. Suto, Y. 1974. Researches on tree diseases in Shimane Prefecture in 1963-1972. Bull. Shimane Pref. For. Exp. Sta. 24:1-40.
8. Suto, Y. 1984. Researches on tree diseases in Shimane Prefecture in 1973-1982. Bull. Shimane Pref. For. Res. Cent. 35:17-26.
9. Takahashi, I., Suzuki, S., Saho, H. 1974. Damage of Dothistroma needle blight on exotic pine species. Proc. Jap. For. Soc. Hokkaido Branch 23:3-6.

Spatial Pattern of *Dothistroma Septospora* Needle Blight in Young *Pinus radiata* Plantations[1,2]

Hernan L. Peredo,[3] Rodolfo Contreras[3,5] and Gaston Vergara[4]

Abstract.—Fifty-one plots were established in 1984 to gather the basic information to model the growth of *Pinus radiata* under different chemical and silvicultural treatments to control *Dothistroma septospora*. The spatial pattern of the damage as part of the model was determined using the disease level measured yearly in every tree, comparing the plots without fungicidal application during three years as control and those with yearly fungicidal applications during the same period as threshold. According to Variance-to-Mean Ratio and Morisita's dispersion indices, the spatial pattern of the defoliation was almost entirely at random independent of either disease level or treatment during the three growing seasons studied. The epidemiological importance of these results is discussed.

At present, vegetal epidemiology is considered a system capable of being divided into sub-systems which can be quantified and also modeled by means of computer programs (11). One of the aforementioned subsystems is the spatial distribution of the disease which can be used to characterize it, to study its relationship with environmental factors, to establish the most adequate technique for its quantification and to determine its relationship with the distribution of the inoculum (3). The analysis of the spatial distribution of diseases has been done in agriculture for almost all types of diseases. In forestry, however, simulators have been developed mainly for stem diseases (11); some disease progress curves for defoliation in conifers are also available (5).

The aim of this study was to determine the spatial distribution of defoliation caused by *Dothistroma septospora* (Dorog.) Morelet in plantations of *Pinus radiata* D. Don during three growing seasons after planting as the first approach to model their growth, including the damages due to pests and diseases.

Materials and Methods

A *P. radiata* plantation established in 1984 in Fundo Las Palmas belonging to Universidad Austral de Chile was used to design a trial to model their growth under different treatments to control the defoliation caused by *D. septospora*. Annual measurements of the level of damage in each individual tree at the end of August and diameter and height growth at the end of November were performed in 51 plots of 400 m^2 containing 45-50 individuals each. The experimental design was a randomized complete block with three repetitions and 17 treatments per block. Treatments included temporal variations in the use of a single dose of fungicide (2.5 kg/ha Antracol-Cobre™ in 40 l water) at the end of November, herbicide (Velpar™ 3.0 kg/ha) and fertilization (NPK+MgB) at the time of planting, pruning and thinning when the dominant height of the trees reached 5.0 m, combinations of all treatments, and controls. Each plot was demarcated using impregnated stakes and labels indicating their number and treatment. Trees were correlatively numbered with metal tags. Moreover, a sketch was designed for each plot showing the position of each tree spaced 2.5 x 2.5 m in the plot (9).

Six plots without fungicide were chosen for this study; three of them corresponded to the absolute control and three to the treatment group without fungicide plus pruning and thinning yet to be done. For comparison, treated plots with annual applications of fungicide and those combining fungicide plus pruning and thinning were used. One of the treated plots was discarded due to poor plant survival caused by factors other than those considered in this study.

Defoliation of each tree during the three years of the study was registered in the sketch as one of the following

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[2] Part of the Project "Modelo de crecimiento para plantaciones jovenes de *Pinus radiata*, sometidas a diferentes tratamientos para el control de *Dothistroma septospora*" financed by Corporacion Nacional Forestal (Chile) and registered by the Direccion de Investigacion y Convenios, Universidad Austral de Chile.

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Table 1.—Spatial distribution of defoliation caused by *Dothistroma septospora* during the first three growing seasons of *Pinus radiata* plantations

Year	DL[a]	Untreated Plots						Treated Plots				
		1	2	3	4	5	6	1	2	3	4	5
1985	1	R[b]	R	R	R	R	R	U	U	U	U	R
	2	R	U	R	R	R	R	-	R	R	R	R
	3	-	R	R	R	R	R	-	-	-	-	-
	4	-	-	-	-	R	-	-	-	-	-	-
1986	1	-	-	A	-	R	-	R	R	U	-	-
	2	R	-	R	-	R	-	R	U	R	R	R
	3	U	R	R	U	R	R	-	-	-	-	R
	4	R	R	R	R	R	R	-	-	-	-	-
1987	1	-	-	-	-	-	-	R	R	R	R	R
	2	R	R	R	R	R	R	R	R	R	R	R
	3	R	R	U	R	R	R	R	R	-	-	-
	4	R	R	-	-	-	-	-	-	-	-	-

[a] Defoliation levels (DL): 1 = 0-25%, 2 = 26-50%, 3 = 51-80%, 4 = 81-100% within each plot.
[b] - = not present, U = uniform, R = random, A = aggregated within each plot.

levels: 1) 0-25%; 2) 26-50%; 3) 51-80% and 4) 81-100%. A grid of 16 squares was overlaid on these data and frequency tables were derived for each level of damage in each plot. The determination of the spatial distribution of the damage was established using the dispersions indices Variance-to-Mean Ratio and Morisita (2). The statistical significance ($p < 0.05$) of both indices was established using Student's t and Chi-square tests, respectively.

Results

The spatial distribution of the damage determined by both indices was almost entirely at random, independent from the treatment and level of damage during the period of the study (table 1). Control plots showed only a few isolated cases of uniform or aggregated distribution, mainly at the lowest level of damage, during the three years of the study. Treated plots, on the contrary, did not show aggregated distribution throughout the study. During the first two years, primarily uniform distributions were determined at defoliation levels 1 and 2. In the third year, however, all plots showed a randomized distribution of damage at all defoliation levels.

Defoliation, however, appeared to have a wider distribution in the control group. All four levels of defoliation were observed in the first two years whilst level of damage 1 disappeared in all plots in the third year. Treated plots did not show defoliation levels 3 and 4 in the first year. In the second year, one plot showed defoliation level 3. After the last year of the trial, defoliation level 3 appeared sporadically whilst none of the plots showed defoliation level 4.

Discussion

The presence of defoliation levels close to 50% in all plots after two years of fungicide treatment and over 50% in two plots after three years of treatment confirmed the chronic characteristic of the disease in Chile (1). Damage intensity caused by *D. septospora* has been commonly described as the percentage of affected green crown assessed visually (7). Damage intensity can also be estimated as the percentage of necrotic needles carrying fruiting bodies of the fungus. These needles, collected at 1.5 m above ground, could be derived from individual trees or a needle pool from which a sample is obtained. The damage intensity thus assessed was not significantly different from the estimate obtained when using the percentage of attacked green crown as long as the sample number was not less than 20 trees. All the previous relationships were obtained in an 8-10 year old *P. radiata* plantation, so the spatial distribution assessed for the first three years of their growth apparently is maintained up to this age. To perform the analysis in 50, 100, or 200 trees did not significantly increase the precision of the estimation of damage intensity (4). A randomized distribution of defoliation not only explains the behavior of the epidemiological parameters mentioned

above but confirms that the estimation of the intensity of the damage caused by *D. septospora* can be done using a transect and by sampling a minimum of 20 trees (4, 7, 8).

The randomized distribution of defoliation indicates that besides the resistance in *P. radiata* induced by age (6) there is also an individual resistance depending on the strength of the plant. This finding could explain why, with permanent inoculum throughout the year (1, 10), not all trees had the same degree intensity of defoliation.

Literature Cited

1. Barudy, J. M. 1980. Estudio fenoldgico de la caida de aciculas de Pino insigne (*Pinus radiata* D. Don) infectades por el hongo *Dothistroma pini* Hulb. Tesis Ing. Forestal. Universidad Austral de Chile, Valdivia. 155 p.
2. Brower, J. E., Zar, J. H. 1977. Field and laboratory methods for general ecology. Wm. C. Brown, Dubuque, Iowa. 226 p.
3. Campbell, C. L., Noe, J. P. 1985. The spatial analysis of soilborne pathogens and root diseases. Ann. Rev. Phytopathol. 23:129-148.
4. Etcharren, R. C. 1984. Algunos aspectos a considerar en la medicion del ataque causado por *Dothistroma pini* Hulb. en Pino insigne (*Pinus radiata* D. Don). Tesis Ing. Forestal. Universidad Austral de Chile, Valdivia. 96 p.
5. Griggs, M. M., Schmidt, R. A. 1986. Disease progress of *Scirrhia acicola* in single and mixed family plantings of resistant and susceptible longleaf pine. p. 5-10. In: G.W. Peterson (tech. coord.). Recent research on conifer needle disease. USDA For. Serv., Gen. Tech. Rep., WO 50. 106 p.
6. Ivory, M. H. 1972. Resistance to Dothistroma needle blight incuded in *Pinus radiata* by maturity and shade. Trans. Brit. Mycol. Soc. 59:205-212.
7. Kershaw, D. J., Gagil, P. D., Leggat, G. J., Ray, J. W., van der Pas, J. B. 1982. Assessment and control of *Dothistroma* needle blight (rev. ed.). N. Z. For. Serv., For. Res. Inst. Bull. 18. 35 p.
8. Lin, C. S., Poushinsky, G., Mauer, M. 1979. An examination of five sampling methods under random and clustered disease distribution using simulation. Can. J. Plant Sci. 59:121-130.
9. Peredo, H., Bello, F., Contreras, R. 1985. Modelo de crecimiento para plantaciones jovenes de *Pinus radiata*, sometidas a diferentes tratamientos para el control de *Dothistroma septospora*. I. Diseno experimental. Universidad Austral de Chile, Facultad de Ciencias Forestales. Serie Technica, Informe de Convenio, Valdivia. 15 p.
10. Rack, K. 1986. Ueber die jahreszeitlichen Entlassung der Konidien von *Dothistroma pini* in *Pinus radiata*-Kulturen des suedlichen Chile. Eur. J. For. Path. 16:6-10.
11. Teng, P. S. 1985. A comparison of simulation approaches to epidemic modeling. Ann. Rev. Phytopathol. 23:351-379.

Dothistroma Needle Blight in Yugoslavia[1]

Dragan Karadžić[2]

Abstract.—*Dothistroma septospora* is one of the most widespread and most dangerous pathogenic fungi in *Pinus nigra* plantations in Yugoslavia. Based on conidial length and the constant presence of its perfect state, it has been concluded that *D. septospora* var. *lineare* occurs in Yugoslavia. Both states of the fungus have been observed, i.e., conidiomata and ascostromata. Conidia are far more significant in the infection process. The critical period for infection is May-July. Of the tested fungicides, the best results have been obtained with "Copper-lime-25" (cupri oxychloride).

Introduction

Intensive afforestation of barren lands and deforested areas has been carried out in Yugoslavia for the last 30 years. Coniferous species have been most widely used for afforestation, above all, species of *Pinus*. In a great many cases pure plantations (monocultures) have been established on large tracts. Out of all pine species, *Pinus nigra* Arn. and *Pinus sylvestris* L. have been the most frequently used. Immediately after the establishment of a plantation, the first damages start to occur, caused by many abiotic and biotic factors, among which diseases and pests are of particular importance.

By detailed examination of *P. nigra* and *P. sylvestris* plantations, some diseases have been observed which had not been detected previously or to which attention had not been paid. In *P. nigra* plantations in Yugoslavia, the greatest damage is caused by *Dothistroma septospora* (Dorog.) Morelet, *Sphaeropsis sapinea* (Fr.) Dyko & Sutton, *Gremmeniella abientina* (Lagerb.) Morelet, *Cenangium ferruginosum* Fr., and, more rarely, by *Lophodermium* spp., *Cyclaneusma niveum* (Pers. ex Fr.) DiCosmo et al., *Cenangium acuum* Cooke & Peck and *Sclerophoma pithyophila* (Corda) v. Hohn. (6,7).

In *P. sylvestris* plantations, the greatest damage is caused by *Heterobasidion annosum* (Fr.) Bres. (especially in plantations on sandy soils), but the fungi causing needle cast [*Lophodermium seditiosum* Minter, Staley & Millar, *L. pinastri* (Schrad.) Chev., *Cyclaneusma minus* (Butin) DiCosmo, Peredo & Minter] also occur very frequently. Damage caused by rust fungi [*Coleosporium sennecionis* (Pers.) Fr., *Melampsora pinitorqua* Rostr. and *Cronartium flaccidum* (Alb. & Schw.) Wint.] occur less frequently. In mountainous regions, greater damage caused by *Phacidium infestans* Karst. (13) and *Lophodermella sulcigena* (Rostr.) Höhn. (11) has been detected recently.

No doubt, *D. septospora* is one of the most widespread and dangerous pathogenic fungi in *P. nigra* plantations in Yugoslavia. *Dothistroma septospora* was discovered in Yugoslavia (Serbia) for the first time in 1955 (9) and today is so widespread that it can be said that all the regions where *P. nigra* grows have been more or less infested. An especially intensive attack occurred at Deliblato Sands and Subotica-Horogos Sands where, in the course of recent years, the fungus has occurred at constant epiphytotic levels.

This paper presents the results of the investigation on the distribution of *D. septospora* in Yugoslavia and its host plants, life cycle, infection mechanism, some morphologic and physiologic characteristics, and the results of some experiments on chemical control in fungicide treatments of *P. nigra* plantations.

Geographic Distribution of *D. Septospora* and Its Hosts in Yugoslavia

Dothistroma septospora is widespread in Yugoslavia (fig. 1). It occurs especially frequently in Serbia, and the most endangered areas are the Deliblato Sands, the Subotica Sands and Juzni Kucaj. In these localities both the anamorph and teleomorph states [described as *Scirrhia pini* Funk & Parker, *Mycosphaerella pini* (Funk & Parker)] have been detected.

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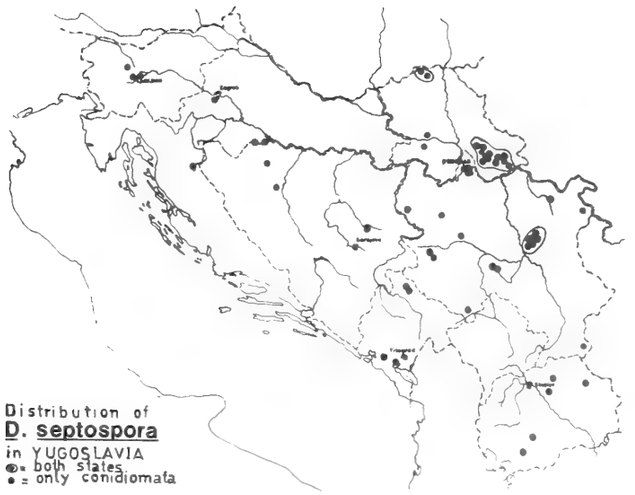

Figure 1.—Distribution of *Dothistroma septospora* inYugoslavia.

Table 1 lists the Pinus species on which this fungus has been detected in Yugoslavia. Of the highly susceptible species, four species (*P. contorta* Dougl., *P. jeffreyi* (Grev.) Balf., *P. ponderosa* Laws., *P. nigra var. maritima* (Ait.) Melville) are exotic plants, and their distribution is relatively limited in Yugoslavia. In Yugoslavia great economic loss is caused by *D. septospora* only in *P. nigra* plantations. Plantings between 5 and 25 years of age are especially endangered. Damage has also been detected on *P. mugo* Turr., but only on park trees. This species is never attacked by *D. septospora* in natural habitats. The main reason for the above is that the ecologic factors prevailing in natural habitats of *P. mugo* are very unfavourable for the development of the fungus. *Dothistroma septospora* has never been recorded in Yugoslavia at the elevations higher than 900 m. As for *P. sylvestris*, it is very resistant to this fungus and even mild infections are very rare.

Table 1.—*Pinus* species that are hosts of *Dothistroma septospora* in Yugoslavia

Host	Susceptibility
P. contorta Dougl.	+++
P. halepensis Mill.	+
P. jeffreyi Grev. Balf.	+++
P. nigra Arn.	+++
P. nigra var. *maritima* (Ait.) Melville	+++
P. ponderosa Laws.	+++
P. mugo Turr.	++
P. sylvestris L.	+

+++ = High susceptibility; ++ = Moderate susceptibility; + = Low (or very low) susceptibility

Disease Symptoms

The first symptoms of the disease on needles infected during the current year appear by the end of September and during October, but they are quite distinguishable during November and December. The infection occurs most often on the previous year's needles, and more rarely on current-year needles.

The first symptoms of the disease are the discoloration of needle tips. The upper half of the needle becomes light green, then turns yellow and finally becomes light brown, while the base of the needle stays green. Most commonly only the portion of the needle above the place of infection changes color. Though this is the most common form of symptom, symptoms may also occur on other parts of the needle. Usually, at places where the fungus penetrates in the first stage, there are dark green bands throughout the needle, but more often in its upper part. Soon after these bands appear, pale reddish and then reddish-brown and brick-red spots develop. Reddish spots and bands are visible at both sides of the needle. The following February the whole needle becomes necrotic and covered with numerous individual or scattered reddish bands and spots. At this time erumpent fruiting bodies of this fungus develop, which destroy the epidermis and erupt to the surface. When conditions are favourable, usually by the end of February, physiologically mature conidia can be observed, though their mass release starts about the end of March.

Some Morphological Characteristics of *D. Septorspora*

In Yugoslavia, both the teleomorph (*S. pini*) and anamorph (*D. septospora*) states of the fungus have been detected. Conidiomata are produced more often than ascostromata. It seems that the anamorph is the parasitic stage of the fungus, whereas the teleomorph is the saprophytic stage. Ascostromata usually are formed only when the needles are completely necrotic, most often on 2- and 3-year old needles.

Conidiomata are always formed within the distinct reddish bands and spots. Conidiomata are linear, subepidermal, erumpent, and black (fig. 2-A, B). Conidia are hyaline, scolecoform, 1- to 5-septate (usually 3-septate) (fig. 2-C, D).

Ascostromata are black, linear, multiloculate, subepidermal, and erumpent (fig. 2-E, G). Ascostromata form on necrotic needles within the reddish bands and spots, but it must be pointed out that these spots and bands are, at this time, somewhat paler than the spots where conidiomata are formed. Asci are cylindrical or clavate, and bitunicate; their apices are rounded; they are eight-spored and separated by pseudoparaphysoids. Ascospores are hyaline, 1-septate, and fusiform (fig. 2-H).

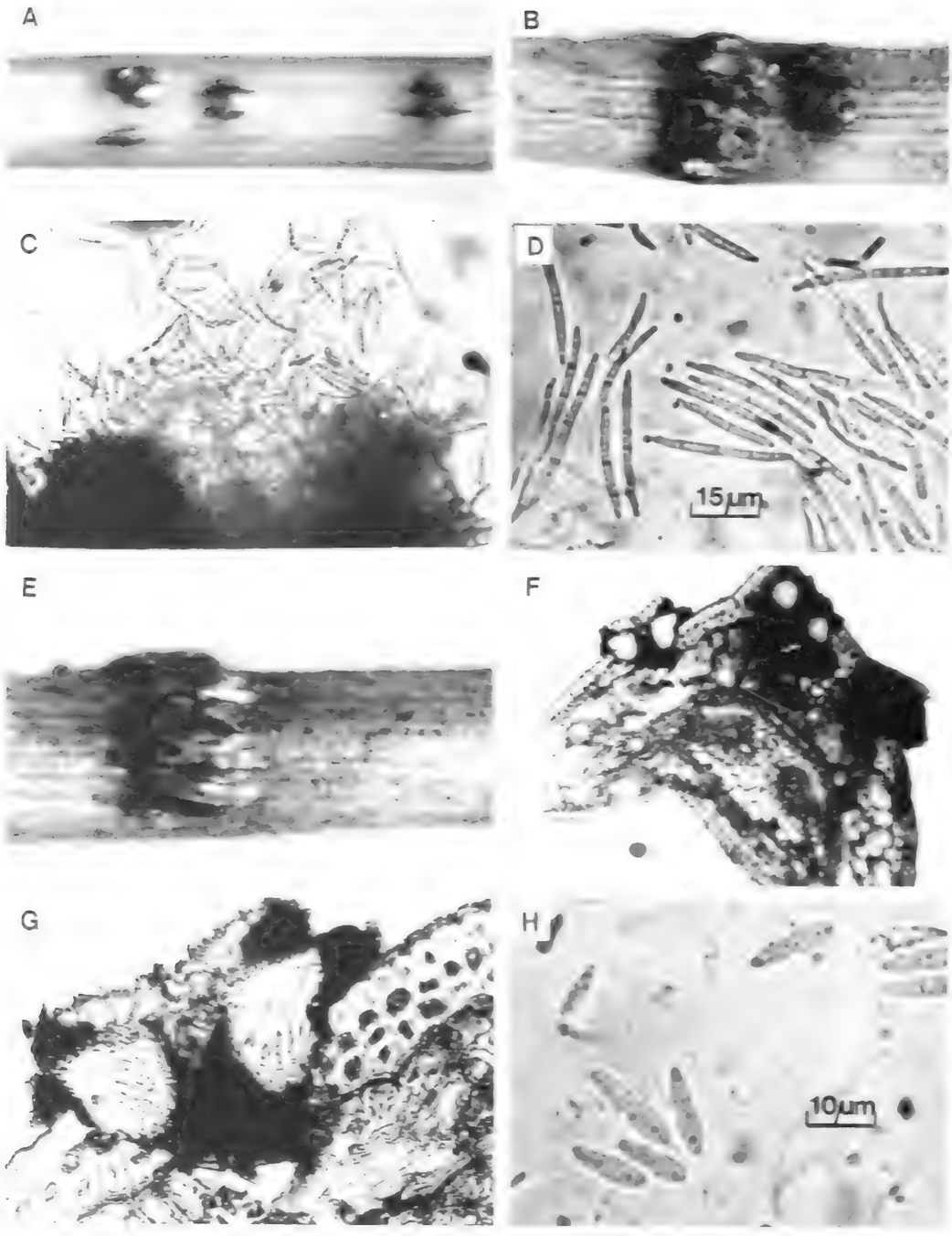

Figure 2.—*Dothistroma septospora*. A. Conidiomata of the fungus on an Austrian pine needle. B. Conidiomata on a Jeffrey pine needle. C. Acervulus with conidia. D. Conidia. E. Ascostromata of fungus on an Austrian pine needle. F. Vertical section of ascostromata. G. An ascostroma with two locules rupturing the host epidermis. H. Ascospores.

Thyr and Shaw (17), on the basis of conidial length, described two varieties of the fungus, *D. pini* var. *linearis* and *D. pini* var. *pini*. Average size of conidia according to the original description was 31.9 x 2.4 µm, for var. *linearis*, and 22.4 x 3.2 µm for var. *pini* . Ivory (5) later described a third variety, *keniensis*, with conidia averaging 28.7 x 2.6 µm. Conidial length was significantly different for all the varieties. The speed of germination also was different (germination was the quickest for the var. *linearis*, and the slowest for the var. *pini*). Sutton (16) listed these varieties as *D. septospora* var. *septospora*, *D. septospora* var. *lineare* and *D. septospora* var. *keniense*.

Based on the length of conidia (table 2) and the mode of germination, the fungus occurring in Yugoslavia is *Dothistroma septospora* var. *lineare* (Thyr & Shaw) Sutton. This variety differs clearly from the var. *pini* by the fact that it always forms its perfect state (ascostromata).

It my opinion that there are no great differences between the varieties *pini* and *keniensis*, and that they are, in fact, the same variety of the fungus, because to describe a variety only on the basis of conidial length may be diagnostically doubtful. A similar opinion was expressed by Gadgil (3).

Table 2.—Size of fruiting bodies and spores of *Dothistroma septospora*

	Length (μm)		Width (μm)		Height (μm)	
	Range	Mean	Range	Mean	Range	Mean
Ascostromata	370-1030	583	198-412	324	160-277	207
Locules	46-127	79	46-144	76		
Asci	33-48	41	6-9	7		
Ascospores	9-17	14	2.4-3.6	3.1		
Conidiomata	290-908	551	205-560	480	205-341	268
Conidia	15-44	31	1.8-3.0	2.5		

The sizes of conidiomata and conidia are affected by host plants; for example, conidial stromata and conidia are smaller on needles of *Pinus ponderosa* than on needles of *P. nigra.*

Life Cycle of *D. Septospora* in Plantations of *P. Nigra*

The results of several years of research can be summarized as follows. *Dothistroma septospora* has a 1-year life cycle. Conidiomata mature at the end of March. Twelve months are required between infection and the new dispersal of conidia capable of infection. Conidiomata rarely may be formed within the year in which infection occurred, but conidia from these are capable of infection only in the following spring. Conidia are dispersed from the beginning of April until the end of October; rarely can they be discharged earlier. Ascospores are dispersed from the second half of June until the end of September. The importance of the conidia in infection is much greater than ascospores because the conidia are dispersed over a longer period and also at a time when environmental conditions suitable for infection are much more favorable. The infection period lasts from the middle of April until the end of August. The critical period for infection is from the beginning of May until the end of July. Most infections occur during May and June; the greatest amount of infection occurs in the first half of June. The length of the incubation period under natural conditions varies depending on the climatic factors, but it is normally from 4 to 6 months. Protection is necessary during critical period for infection, i.e., from the beginning of May until the end of July.

Infection of *P. Nigra* Needles by *D. Septospora*

For infection, 2- and 4-year-old nursery transplants were used. The plants were inoculated by spraying the needles with a water suspension of conidia from 3-week-old cultures of *D. septospora* on 2% malt agar. After inoculation the plants were incubated for 7 days in plastic bags in a greenhouse mist chamber (temperature 20 C, relative humidity 100%). Uninoculated plants were used as a control. Observations were made 24, 48, 62 and 192 hours after inoculation. Further observations were continued every 15 days until the first symptoms of the disease appeared.

Scanning electron microscopy was used to follow the course of penetration and infection for surface phenomena.

The greatest number of *D. septospora* conidia germinated on the needles of Austrian pine after 48 hours (individual conidia started to germinate after 8 hours) by forming one to three germ tubes (fig. 3-A, E). Most frequently, three germ tubes were formed, two at the ends and one in the center (fig. 3-E). Germ tube development on the needle surface varied and was more or less irregular. Growth of germ tubes was directed positively towards the stomata but some showed no evidence of stomatal attraction (fig. 3-F). After 8 days appressoria-like structures were formed in the stomata (fig. 3-G). The infection hypha started from the center of these appressorium-like structures and penetrated the guard cells, then colonized the mesophyll. Eight days after inoculation, numerous secondary conidia formed on the surface of the needles (fig. 3-H). Within the mesophyll, the mycelium penetrated both intercellularly and intracellularly, occupying even the endodermis and resin canals. Hyphae have never been observed in the vascular tissues of the needles. The first symptoms on the inoculated needles developed after 4-8 weeks, while the first fructifications (conidiomata) developed after 3.5 - 4.5 months. Direct penetration of the epidermis was never observed.

Our observations generally agree with those of Gadgil (3) on infection of *P. radiata* needles by *D. pini.*

In our experiments all attempts at infection of 2-year-old *P. sylvestris* were unsuccessful.

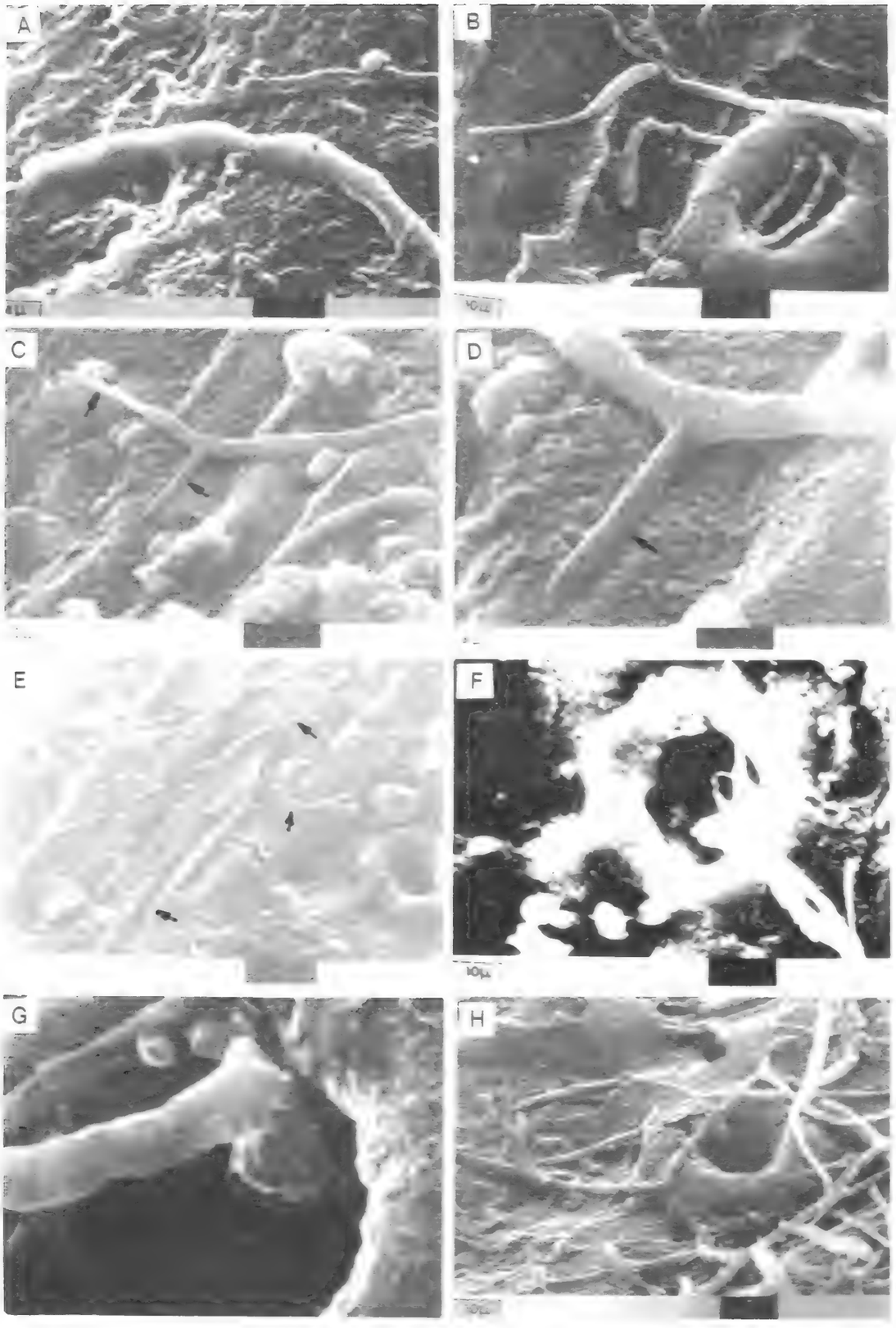

Figure 3.—*Dothistroma septospora*. A. Conidium of the fungus on an Austrian pine needle. B,C,D,E. Germinating conidia. F. Direct penetration of the germ tube through a stoma. G. Appressorium-like structure formed in the antestomatal chamber. H. Secondary conidia developing on mycelium on the surface of the needle.

The Effect of Some Ecological Factors on Reproductive Organ Germination and Mycelial Growth of *D. Septospora*

Conidia of *D. septospora* germinated on water agar within the temperature range of 5 to 30 C, with an optimum of 22 C. The germ tubes grew most quickly at 22 C and reached an average length of 72 µm after 22 hours. Conidial germination began after 8 hours and the highest percentage germinated within the first 22 hours. Conidia germinated with two or three germ tubes. Optimal temperature of ascospore germination was 22 C, minimum 5 C, and maximum 30 C. Percentage of ascospore germination was lower than the percentage of conidial germination. Ascospores germinated with two germ tubes, i.e., one was formed at the end of both cells. These germ tubes grew more slowly than those formed from conidia.

Optimum relative humidity for conidial germination was 100%, minimum 88% (at this relative air humidity only individual conidia germinated, and they did so only after 8 days). At a relative humidity of 98.8% to 100%, germ tubes grew in all directions throughout the surface of the needle, without any regularity of growth in the direction of the nearest stoma. At a relative humidity of 96.8 to 98%, most germ tubes (about 80%) grew in the direction of the nearest stoma. At a relative humidity of 96.8 to 100%, secondary conidia were produced on the mycelium after 8 days.

The best germination of conidia was obtained on acid media (pH 6). Conidia germinated within a pH range of 2.5 to 9. The change of substrate acidity did not affect the percentage of conidial germination except at extremely high acidity or alkalinity.

The optimum temperature for mycelial growth was 20 C, minimum 3 C, and maximum 29 C. Growth of mycelium was very slow and at optimal temperature, on malt extract agar, it amounted to between 0.9 and 1.1 mm per day, depending on the isolate. On a 2-week old colony, within the temperature range of 10 (15) to 25 C, numerous conidia were formed. Of all the tested nutritive media, *D. septospora* grew best on malt extract agar.

Most researchers agree that conidia germinate within the temperature range of 5 to 30 C, but differ regarding the optimum temperature. Ivory (5) reported the optimal temperature for conidial germination was 18 C, Ito et al. (4) 20 C, Peterson (14) 24 C (germination was optimum at 22 C), Lasca et al. (10) 18 C, Sheridan and Yen (15) 17 C, Lindberg (12) 26 C (maximum temperature according to this author was 35 C). The results of our studies agree generally with the results of Peterson (14). The differences probably can be explained by the existence of different strains or races of the fungus.

The impact of *D. Septospora*

In Yugoslavia, *D. septospora* causes economic losses in *P. nigra* plantations. Plantations between 5 and 25 years of age are especially endangered. *Dothistroma septospora* infects the previous year's needles, and more rarely the needles of the current year. Increment loss occurs when defoliation is more than 40%. The loss is first expressed in the decrease of height increment. The growth of trees where current-year needles are also infected is significantly retarded; they are physiologically weakened and, most often, after 4-5 years of repeated attacks they die. This phenomenon has been most obvious at the Deliblato Sands (the south-eastern fringe of the Pannonian Plain). One of the reasons for intense infections in *P. nigra* plantations of the Deliblato Sands is that during the period of mass release of conidia, the regimes of rainfall and temperature are very favourable for infection. Losses in this region have been increased by the fact that *Sphaeropsis sapinea* occurs together with the fungus *D. septospora*. *Sphaeropsis sapinea* kills the new shoots and the second-year needles are infected by *D. septospora*, so that some trees are left completely without assimilative organs. Consequently, the trees in the plantation die usually starting from their tops (die-back). The phenomenon occurs on trees between 20 and 30 years old, and it has been becoming more epidemic in character. In some localities *D. septospora* kills natural progeny of *P. nigra*, thus preventing its natural regeneration.

Control

Considering the great losses caused by *D. septospora* in *P. nigra* plantations, experiments with various protective fungicides were begun. Previous preliminary investigations showed that the greatest percentage of infection occurred during May and June, so to be economical, two treatments (beginning of May and beginning of June) were used. The first treatment was aimed to protect second-year needles (the most susceptible needles), and the second was to protect newly-formed first-year needles. The experiments were carried out at two localities in *P. nigra* plantations of the Deliblato Sands. The experiments at Baraka lasted from 1976 to 1980, and those at Dragicev Hat from 1977 to 1983.

The following fungicides were used: "Orthocid S-50" (Captan), "Copper-lime-25" (copper oxychloride), "Cineb S-65," a 2:1 combination of copper oxychloride and Cineb S-65, and "Benlate."

Control efficacy was estimated in three ways: by measuring the diameter at breast height of treated and control trees to the nearest 0.1 mm, by measuring the height increment to the nearest 1 cm, and by the visual rating of the healthy appearance of the tree on a 1 to 5 index.

The results of the investigation showed:

- There were no significant differences in diameter increments between the treated and control trees.
- There were significant differences between the average height increments of treated and control trees. The same differences occured between trees treated with "Copper-lime-25" and "Cineb S-65" when compared to the control trees, whereas there no appreciable differences between the trees treated with "Orthocid S-50" and the control.
- There were significant differences in the evaluation of the healthy appearance of the trees treated with fungicides and the control trees. There were considerable differences between the trees treated with "Copper lime-25" and "Benlate," as compared to the control trees, whereas these differences were not expressed in case of trees treated with "Cineb S-65" and "Orthocid S-50" when compared to the control trees.
- In all the experiments "Copper Lime-25" rendered the best results and protection. The protection was satisfactory if the treatment was carried out twice a year during the critical period of infection but, because of economic reasons, protection should be applied at the beginning of May and June every third year in 5- to 20-year-old plantations.

Laboratory investigations showed that all tested fungicides strongly inhibited the germination of *D. septospora* conidia.

The greatest inhibition of mycelial growth was produced by "Benlate," and inhibition was rather pronounced with "Copper lime-25" and "Cineb S-25." The least inhibition was caused by "Orthocid S-50" which, in small amounts, stimulated mycelial growth.

Benlate showed the greatest toxicity to *D. septospora* mycelium. It was lethal to the fungus at a concentration of 0.01% with an exposure of 1 minute. Of the remaining fungicides, good results were obtained with "Copper lime -25". (For more details, see 8).

Literature Cited

1. Arx, J. A. von. 1983. *Mycosphaerella* and its anamorphs. Proc. Koninklijke Nederlandse Akademie van Wetenschappen, Series C, 86:15-54.
2. Funk, A., Parker, A. K. 1966. Scirrhia pini n. sp., the perfect state of *Dothistroma pini* Hulbary. Can. J. Bot. 44:1171-1176.
3. Gadgil, P. D. 1967. Infection of *Pinus radiata* needles by *Dothistroma pini*. N. Z. J. Bot. 5:498-503.
4. Ito, K., Zinno, Y., Suto, Y. 1975. *Dothistroma* needle blight of pines in Japan. Bull. Jap. Gov. For. Exp. Sta. 272:123-140.
5. Ivory, M. H. 1967. A new variety of Dothi*stroma pini* in Kenya. Trans. Br. Mycol. Soc. 50:289-297.
6. Karadžić, D. 1983. Bolesti četina crnog bora (Pinus *nigra* Arn.). Zaštita bilja 34:329-342.
7. Karadžić, D. 1987. Uticaj patogene mikoflore na propadenje i sušenje stabala u kulturama Pinus vrsta. Šumarstvo 5:89-106.
8. Karadžić, D. 1987. Efikasnost nikih fungicida u suzbijanju gljive *Dothistroma pini* Hulbary u kulturama crnog bora. Zaštita bilja 38:15-31.
9. Krstić, M. 1958. Nezabeležene fitopatološke pojave u rasadnicima i sumama Srbije. Zaštita bilja 45:75-79.
10. Lasca, C. C., Figueiredo, M. B., Namekata, T. 1974. Dothistroma pini Hulbary variedade *Pini* Thyr et Shaw. em *Pinus pinaster* Ait. no Estado de S. Paulo. O. Biologico 40:267-270.
11. Lazarev, V. 1983. Bolesti iglica bijelog bora (P*inus sylvestris* L.). Zastita bilja 34:265-274.
12. Lindberg, G. D. 1950. A needle blight of ornamental pines caused by Dothistroma *pini*. M.S. Thesis, Oklahoma State University, Stillwater, OK. 46 p.
13. Marinković, P., Karadžić, D. 1983. Pojava novih opasnih patogena u kulturama crnog i belog bora u Jugoslaviji *Gremmeniella abietina* (Lagerb.) Morelet i. *Phacidium infestans* Karst. Šumarstvo 5-6:3-12.
14. Peterson, G. W. 1981. Pine and Juniper Diseases in the Great Plains. USDA For. Serv. Gen. Tech. Rep. RM-86, 47 p.
15. Sheridan, J. E., Yen, C. C. 1970. A note on the effect of temperature and relative humidity on the germination of conidia of a New Zealand isolate of *Dothistroma pini* Hulbary. N. Z. J. Bot. 8:658-660.
16. Sutton, B. C. 1980. The Coelomycetes. Commonwealth Mycological Inst., Kew, England. 696 p.
17. Thyr, B. D., Shaw, C. G. 1964. Identity of the fungus causing red band disease on pines. Mycologia 56:103-109.

A Needle Blight of *Pinus Strobus* : History, Distribution, Signs and Symptoms[1,2,3,4]

T. A. Dreisbach and W. Merrill[5]

Abstract.—A needle blight of *Pinus strobus* L., cause unknown, has been observed in the northeastern United States for at least 80 years. This blight is characterized by a die-back of current-season needles, beginning in mid-summer. A complex of fungi is associated with this blight, including a previously undescribed hystereaceous fungus which infects current-year needles in June and July and forms hysterothecia the following year.

Introduction and History

For many years, reports have appeared pertaining to a needle blight of eastern white pine, *Pinus strobus* L. This blight has been described as a die-back of current-season needles, giving a red or scorched appearance to affected trees. In 1908 Dana published the first report of this disease (11) and noted that it occurred from Maine to Pennsylvania. Campana (7) summarized the information from 1894 to 1952 pertaining to a "red needle blight" and noted that many causal agents had been suggested, including drought, root mortality, frost, adverse climate, industrial pollution, and parasites.

Campana (7) believed the root rot fungus, Corticium galactinum (Fr.) Burt, could be responsible for the blight. He inoculated *P. strobus* seedlings with this pathogen but was unable to reproduce the symptoms. He concluded that this fungus did not contribute to blight symptoms, and that the causal agent was abiotic. Baldwin (1) reported needle blight of *P. strobus* in New Hampshire and postulated that abundant rainfall followed by sudden drought was responsible.

In 1960 Linzon saw orange-red tips on current-year needles of eastern white pine in Ontario, Canada and named the condition "semimature-tissue needle blight" (SNB) (18). Fungi fruiting 3 to 4 weeks after SNB outbreaks were considered saprophytes. In 1962 Linzon identified *Hypoderma desmazierii* Duby and *Cenangium acuum* Cook & Peck fruiting on one-year-old, attached necrotic needles, and a *Lophodermium* sp. (either *L. pinastri* (Schrad. ex Hook.) Chev. or *L. nitens* Darker) fruiting on fallen needles (19). Inoculation studies with *Lophodermium* sp. and *C. acuum* did not produce the SNB symptoms on current-year needles. However, *H. desmazierii* was not used for inoculations due to insufficient amounts of inoculum. Linzon continued to study SNB throughout the 1960s, including attempts to produce the symptoms with ozone fumigation, but never identified a causal agent (20, 21). Also in the 1960s Banfield reported fungi to be responsible for the needle blight in the northeastern United States. He identified a *Lophodermium* sp. that caused yellow-orange lesions that later developed into brown/reddish-brown lesions on first-year needles, with casting of first-year needles from July throughout the year. Apothecia of a *Lophodermium* sp. developed on fallen or dead needles, or occasionally developed the following spring on dead tips of attached needles (2). Later he again associated small yellow spots on white pine needles with a *Lophodermium* sp. (3). Then in 1963 he described symptom progression over the course of a year which included yellow spots developing on current-year needles in early July, leading to yellowing and browning of needles with the imperfect stage of a needlecast fungus appearing in the fall. The following spring he identified *Hypoderma desmazierii* fruiting on the browned needles (4).

Since 1983 the blight has been noted to occur from West Virginia to Maine, and similar symptoms have been noted on *P. strobus* in North Carolina and Wisconsin. In 1983 a needle blight of *P. strobus* was observed in the Acadia National Park, Maine. Although it was not certain that this was the same blight

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[2] Contribution No. 1779, Department of Plant Pathology, The Pennsylvania State University.

[3] Portion of a thesis submitted by the senior author in partial fulfillment of the requirements for the M.S. degree, The Pennsylvania State University.

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mentioned in the literature, symptoms were strikingly similar. From a distance affected trees appeared brown when compared with unaffected trees. A variety of symptoms was observed on current-year needles, including chlorotic mottle, chlorotic spots, tip reddening, and tip necrosis. Needles in the same fascicle often showed different degrees of symptoms.

Participants at a workshop in Acadia National Park in August, 1986 concluded that the symptoms were due either to ozone damage or was SNB (5). However, current-year needles collected at that time bore fruiting bodies of a Leptostroma sp. Lepto*stroma* spp. are the asexual stages of several needlecast fungi.

The symptoms generally caused by needlecast fungi are quite similar to the symptoms described for the blight, including yellow spots and red necrotic tips (6). This fact, along with the presence of the *Leptostroma* stage on current-year needles as early as August, suggested that needlecast fungi may have infected the needles during shoot elongation and thus could be playing a role in the blight.

The objectives of this study were to identify fungi associated with the observed blight of *P. strobus* , and to determine the time of infection by these fungi.

Materials and Methods

To determine which fungi were present on needles we surveyed visibly affected *P. strobus* in May, July and October of 1987 and June and September of 1988 on sites in Pennsylvania, New Hampshire, Vermont, and Maine, including Acadia National Park. By direct observation of fruiting bodies and spores present on the needles, we identified fungi on the 1986, 1987, and 1988 needle complements.

In addition, we conducted intensive sampling of foliage in Acadia National Park during the summer of 1988 to determine when the associated fungi were infecting. Four previously-symptomatic trees were selected, permanently marked, and observed for symptom development. Three trees were located in Acadia National Park (C-1, C-6, C-11), and the fourth tree was located about 25 miles north near Dalton, Maine (E-1). In mid-June branches were tagged, usually one branch each on the southwest and northeast aspects of each tree. At this time needles were just beginning to emerge from the fascicle sheaths. Beginning 13 June 1988 these branches were sampled twice weekly until 4 August 1988, during the period of needle elongation, when it was thought that the previously observed fungi might be infecting. Needles with different symptom types as well as green, asymptomatic needles were sampled. In June the needles had barely emerged from the fascicle sheath, and thus entire fascicles were collected. After needle length reached 2.0 cm, individual needles were sampled. In the laboratory 1 cm sections were cut from symptomatic areas which which were then cut again in half; one half was plated out on 2% acidified malt agar (17), and the other half was fixed in formalin-acetic acid-alcohol (FAA) (9) for later anatomical studies. Results of anatomical studies are not included in this report. Plates were examined weekly, and resulting fungal growth was transferred to potato dextrose agar slants (15 g agar, 20 g dextrose, 1 L potato broth) and transported to Pennsylvania for further study at the end of the field season.

Results

In the 1987 study, fungi were identified on both the 1986 and 1987 needle complements. One of the fungi most frequently found on 1986 needles was tentatively identified as a *Bifusella* sp. Usually only one or two needles per fascicle bore fruiting bodies. The long, shiny, black hysterothecia resembled the needlecast fungus *B. linearis* (Pk.) v. Hohn. However, the fungal fruiting body developed subepidermally, and asci measured 95 x 15.5 µm, each containing 8 rod-shaped ascospores, averaging 23 x 6 µm. Ascospores did not exhibit the distinctive bifusiform shape described for *B. linearis* but more closely resembled the size and shape of ascospores described for the needlecast fungus *Meloderma (= Hypoderma) desmazierii* (Duby) Darker (13). Dr. David Minter, International Mycological Institute, has determined that this *Bifusella*-like Ascomycete (BLA) is a previously undescribed species, and its genus is uncertain (D. Minter, pers. comm. 1988).

Another Ascomycete fruiting on 1986 needles resembled a *Hemiphacidium* or *Phacidium* sp.; however, this fungus was not as prevalent in 1988 on the 1987 needles. Several Fungi Imperfecti were found on both 1986 and 1987 needles, including Hendersoni*a pinicola* Wehmeyer, a *Septoria* sp., a *Leptostroma* sp. and *Truncatella truncata* (Lev.) Steyaert. In addition, *Lophodermium nitens* was ubiquitous on needles lying on the duff.

During the 1988 study symptoms developed on three trees: C-6, C-11 and E-1. Three symptom types developed: chlorotic spots, necrotic tips, and chlorotic spots with necrotic centers. Table 1 shows the dates when each symptom was observed for each tree.

The fungi identified in 1988 on the 1987 complement were: the BLA on all four trees (but very sparse on C-1); *H. pinicola* on C-6, C-11, and E-1; *T. truncata* on C-6 and E-1; *Leptostroma* spp. on E-1, C-11, C-1; and a *Septoria* sp. and the *Hemiphacidium*-like Ascomycete on C-11.

Table 1.—Pinus strobus needle blight symptom types and the date of appearance on the 1988 needle complement

Tree	Symptom type	Date (1988)
E-1	chlorotic spot	June 28 - August 4
	necrotic tip	July 8 - August 4
	chlorotic spot/necrotic center	July 22 - August 4
C-1	chlorotic spot	July 1*
C-6	chlorotic spot	July 29 - August 4
	necrotic tip	July 29 - August 4
	chlorotic spot/necrotic center	August 1 - August 4
C-11	chlorotic spot	August 1 - August 4
	necrotic tip	August 4

* one date only

Single spores of *H. pinicola*, *T. truncata* and the *Septoria* sp. were used to generate reference cultures for comparison with fungi isolated from current-year needles. When needles containing hysterothecia of the BLA were suspended over agar, ascospores were ejected and produced germ tubes on water agar, acidified malt agar, and potato dextrose agar. However, transfers were only successful when several spores were transferred together to potato dextrose agar. Even these cultures were very slow growing, mounding up and reaching a diameter of less then 1 cm after 1 month. As these cultures aged, the medium became dark colored and cultures ceased growth. Subsequent transfers were unsuccessful.

Fungi isolated from 1988 needles were identified on the basis of conidia formation and by comparison of cultural characteristics with those of cultures obtained from spores of fungi fruiting on 1987 needles. Fungi identified from from the isolations from 1988 needles include: *H. pinicola*, *T. truncata*, and *Septoria* spp. Several *Leptostroma*-like cultures were obtained. Cultures resembling ascospore cultures of the BLA were also obtained. Two other fungi frequently isolated were a black, yeast-like fungus, and a white, appressed, nonsporulating fungus. Table 2 lists the identified fungi isolated from 1988 needles.

Table 2.—Fungi isolated from the 1988 needle complement of *Pinus strobus*

Tree	Symptom[a]	Fungus	Date isolated (1988)
E-1	A	*Hendersonia*	June 13 - August 4
		Black yeast	June 13 - August 4
		Leptostroma	July 8 - July 18
	CS	*Leptostroma*	July 1 - August 1
	NT	BLA	July 8 - July 25
		Hendersonia	July 18 - August 4
		Black yeast	July 22 - August 4
	CS/NC	Black yeast	August 4
C-1	A	Black yeast	June 24 - July 22
C-6	A	Black yeast	June 13 - July 18
		White nonsporulating	July 18 - August 4
	CS	White nonsporulating	July 29 - August 4
	NT	*Truncatella*	July 29 - August 1
		Septoria	July 29 - August 4
		Black yeast	July 5 - July 29
		Hendersonia	August 1 - August 4
		White nonsporulating	August 1
	CS/NC	White nonsporulating	August 1
C-11	A	Black yeast	June 21 - June 24
		Hendersonia	June 24
		Leptostroma	July 18
	NT	*Hendersonia*	August 1 - August 4
		Truncatella	August 1

a = A: asymptomatic
CS: chlorotic spot
NT: necrotic tip
CS/NC: chlorotic spot with necrotic center

Discussion

Symptoms.—Symptom development varied on each of the four trees observed in the summer of 1988. E-6 was the most symptomatic tree throughout the study, exhibiting all three symptom types. The same symptoms developed in the same order on C-6; however, symptom development did not begin until 4 weeks later than on E-1. On one date (July 5) C-6 exhibited necrotic tips which were the result of feeding by the pine chafer, *Pachystehus oblivia* (Horn). Necrotic tips were not evident again until July 29. Chlorotic spots and necrotic tips developed on C-6 toward the end of the study (August 1 - August 4). C-11 also developed chlorotic spots, and the necrotic tips; however, this did not occur until the last two sampling dates. It is not known if this tree eventually exhibited the chlorotic spots with necrotic centers such as appeared on C-6 and E-1. C-1 was an asymptomatic tree in 1988, developing chlorotic spots on one day only (July 1), and not exhibiting symptoms thereafter. These evanescent chlorotic spots may have been due to insect feeding.

Needlecast fungi.—Six species of needlecast fungi have been reported on *P. strobus*: *Bifusella linearis*, *Meloderma (= Hypoderma) desmazierii*, *Lophodermium nitens*, *L. pinastri* , *L. pini-excelsae* Ahmad, and *L. durilabrum* Darker (16, 22, 26). In this study *L. nitens* fruited on fallen needles, and a fungus resembling *B. linearis* fruited on necrotic areas on 1-year-old attached needles.

Lophodermium nitens was nearly always present in the duff of *P. strobus*, and thus could be the sexual stage of the *Leptostroma* spp. which were isolated from current-year needles. This cannot be confirmed, however, since descriptions of *Lophodermium* spp. in culture are lacking or inadequate (22), and cultures of *Leptostroma* spp. exhibit highly variable morphology, even among isolates derived from a single fruiting body (25). *Lophodermium nitens* is believed to be a saprophyte of fallen or senescing needles (22), and thus one would not expect *L. nitens* to be present in green, attached needles, as the *Leptostroma* sp. isolated in this study. However, the fact that *L. nitens* sporulates only on fallen needles is not conclusive evidence that this fungus is not a pathogen. As for most needlecast fungi, the life history of *L. nitens* has never been thoroughly investigated. Furthermore, endophytic *Leptostroma* spp. have been isolated from *Pinus* spp. in the Pacific Northwest (8). Therefore, we can draw no conclusions regarding the pathogenicity of the *Leptostroma* spp. isolated from *P. strobus* during this study.

The BLA occurred on all four trees during the 1988 study, and occurred throughout Vermont, New Hampshire, and Maine, as well as in Pennsylvania. It was also collected from *P. strobus* Christmas trees in West Virginia (Merrill and Wenner, unpublished 1986). From field observations it appears the hysterothecium of this fungus develops subepidermally on 10- to 11-month-old needles during the early spring and is not noticeable until May. Ascospores mature in June and July and during wet periods the fruiting bodies split longitudinally, releasing the ascospores which presumably infect current-year needles during shoot elongation. After ascospore release, needles bearing fruiting bodies are cast, usually by late July or August. If an observer is not in the field during the short period of fruiting body maturation and sporulation, the signs of infection by this fungus could easily be overlooked. Furthermore, the similarities of the hysterothecia with those of *B. linearis* easily lead to misidentification. *Bifusella linearis* (= *Hypoderma linearis*) has been reported on *P. strobus* (14,24), but has usually not been considered an aggressive pathogen. Since in some respects the BLA resembles *Meloderma (= Hypoderma) desmazierii*, this may be the fungus described by Banfield (4) as associated with and causing needle blight of *P. strobus* in Massachusetts and by Linzon in Ontario, Canada (19).

In our studies the BLA was isolated from necrotic tips of E-1 from July 8 to July 25. Since this was rather early during needle elongation, this fungus probably is pathogenic on *P. strobus* .

Other fungi.—During the 2 years of this study *Hendersonia pinicola* was found fruiting on 1-year-old *P. strobus* needles from Vermont, New Hampshire, and Maine, and was recovered from current-year needles of all three symptomatic trees during the 1988 isolation studies. *Hendersonia pinicola* was at first isolated from asymptomatic fascicles at the beginning of the 1988 isolations, from June 13 to June 24. However, this fungus probably had not infected the needles at that time. On those sampling dates the needles had barely emerged from the fascicle sheaths, and entire fascicles were sampled. It is possible that spores had lodged under the fascicle sheath and were not killed during surface sterilization. Further, when entire needles were sampled (June 28 and thereafter), *H. pinicola* was isolated only from necrotic tips near the end of the study (July 25-August 4). Although *H. pinicola* has been considered a weak pathogen or saprophyte (14), some believe it may act as an antagonist of certain needlecast fungi (13) or act as a pathogen on *Pinus contorta* var. *latifolia* Engelm. (23). At the present time the role of this fungus on *P. strobus* is unknown.

Two fungi that were consistently isolated from 1988 needles remain unidentified. A fungus which grew as a black yeast in culture was isolated from asymptomatic needles of all trees, as well as from necrotic tips of E-1 and C-6, and on one occasion (August 4) from chlorotic spots with necrotic centers

of E-1. As with *H. pinicola*, isolations from asymptomatic needles during the early days of the study (June 13 - June 24) may be the result of spores lodged in the fascicle sheath which escaped surface sterilization. Numerous species of approximately 30 fungal genera are known to grow in culture as black yeasts (12). The majority of these fungi resemble species of *Aureobasidium* (= *Pullularia*), but can be differentiated by several morphological characteristics, chiefly by method of conidial formation (12). Virtually all references to species of *Aureobasidium* or *Pullularia* on conifer needles, such as the report of *Aureobasidium pullulans* (de Bary) Arn. on ozone-injured *P. strobus* needles (10), are suspect. If reference cultures of such fungi are unavailable for confirmation of identification, reports such as this must be regarded as unproven.

A white nonsporulating hyphomycete was consistently recovered from all symptom types and from asymptomatic needles of C-6, beginning July 18. This culture did not match the morphological characteristics of any cultures derived from fruiting bodies present on 1-year-old needles. Until these two fungi are positively identified, no presumptions can be made as to their roles, if any, in the observed needle blight.

The *Septoria* sp. fruiting on 1-year-old needles was isolated only occasionally from necrotic tips of C-6. *Septoria spadicea* Patt & Charles has been associated with a needle blight of *P. strobus* and is considered a parasite (14). Two *Pestalozzia* spp. have been associated with blighted needles of *P. strobus* (14); however, this genus has since been segregated into five genera on the basis of conidial septation (13). In this study a species of one of those segregates, *Truncatella truncata*, was observed on 1-year-old needles and isolated from necrotic tips of current-year needles. Funk reports that this fungus is a secondary invader of *Tsuga heterophylla* (Raf.) Sarg. Presently the pathogenic capabilities of the *Septoria* sp. and *T. truncata* on *P. strobus* are unknown.

Conclusions

A complex of fungi has been found associated with a needle blight of *P. strobus* in the northeastern United States. A previously undescribed hysteraceous fungus fruits on 1-year-old needles and can be isolated from symptomatic first-year needles as early as July. Other fungi associated with this blight include *Hendersonia pinicola*, *Truncatella truncata*, *Leptostroma* spp., a *Septoria* sp., a black yeast and a white, nonsporulating hyphomycete.

Koch's postulates must be completed to determine which, if any, of these fungi is pathogenic. For the Fungi Imperfecti, conidia can easily be produced in vitro. In this study *H. pinicola*, *T. truncata*, and the *Septoria* sp. sporulated readily on potato dextrose agar within several weeks. However, completion of Koch's postulates with the BLA is impossible at this time. If the life cycle of this fungus is similar to those of other hysteriaceous needlecast fungi, only ascospores are infectious. Since cultures of the BLA are short-lived and do not form ascomata, at present only ascomata from naturally-infected needles can be used as a source of ascospores. However, these needles cannot be used to complete Koch's postulates, since these infected needles often bear fruiting bodies of species of Hendersonia, *Truncatella,* and *Septoria* along with the BLA. Therefore, it would be impossible to separate out the effects of each individual fungus. A method must be devised to collect mature ascospores from the hysterothecia of needlecast fungi and to utilize these ascospores as inocula under controlled environmental conditions. Only when these methods are devised will we begin to understand the life histories of the various needlecast fungi and their interactions with other needle-inhabiting fungi and their hosts.

Literature Cited

1. Baldwin, H.I. 1954. Needle blight in eastern white pine. Plant Dis. Rep. 38:725-727.

2. Banfield, W.M. 1960. Lophodermium needle cast of the eastern white pine. [Abst.] Phytopathology 50:628.

3. Banfield, W.M. 1962. Experimental evidence of pathogenicity for the *Lophodermium* associated with dead needles of eastern white pine. [Abst.] Phytopathology 52:2.

4. Banfield, W. M. 1963. Comparative development of the Hypoderma and Lophodermium needle casts of eastern white pine. [Abst.] Phytopathology 53:870.

5. Bennett, J.P., Anderson, R.L., Campana, R., Clarke, B.B., Houston, D.B., Linzon, S.N., Mielke, M.E., Tingey, D.T. 1986. Needle tip necrosis on eastern white pine in Acadia National Park, Maine. Results of a workshop to determine possible causes. Unpub. mimeo. rept. U.S. Nat. Park Service, Bar Harbor, Maine. 6 p.

6. Boyce, J.S. 1940. Needle cast diseases of conifers caused by *Bifusella*, *Hypoderma*, *Hypodermella*, and *Lophodermium*. p. 87-89. In: H. I. Baldwin (ed.), Important Tree Pests of the Northeast. Publ. for N.E. Section, Soc. Am. Foresters by Evans Printing Co., Concord, NH. 189 p.

7. Campana, R. 1952. White pine needle blight and *Corticium galactinum* (Fries) Burt. Ph.D. dissertation, Yale University, New Haven, Connecticut. 128 p.

8. Carroll, G.C., Carroll, F.E. 1978. Studies on the incidence of coniferous needle endophytes in the Pacific Northwest. Can. J. Bot. 56:3034-3043.

9. Clark, G. ed. 1973. Staining Procedures. Williams & Wilkins Co. Baltimore. 418 p.

10. Costonis, A.C., Sinclair, W.A. 1972. Susceptibility of healthy and ozone-injured needles of *Pinus strobus* to invasion by *Lophodermium pinastri* and *Aureobasidium pullulans*. Eur. J. For. Path. 2:65-73.

11. Dana, S.T. 1908. Extent and importance of the white pine needle blight. USDA For. Serv. unnumbered circular. 4 p.

12. De Hoog, G.S., Hermanides-Nijhof, E.J. 1977. Survey of black yeasts and allied fungi. In: The black yeasts and allied hyphomycetes. Studies in Mycology 15:178-221.

13. Funk, A. 1985. Foliar Fungi of Western Trees. Canadian Forestry Service, Pacific Research Centre, Victoria, B.C. 159 p.

14. Hedgecock, G.G. 1932. Notes on the distribution of some fungi associated with diseases of conifers. Plant Dis. Rep. 16:28-42.

15. Hirt, R.R. 1959. *Pinus strobus* L.: A literature review and discussion of its fungous diseases in North America. SUNY Coll. Forestry at Syracuse Univ. Tech Pub. No. 82, 90 p.

16. Hunt, R.S., Ziller, W.G. 1978. Host-genus keys to the Hypodermataceae of conifer leaves. Mycotaxon 6:481-496.

17. Kistler, B.R., Merrill, W. 1978. Etiology, symptomology, epidemiology, and control of *Naemacyclus* needlecast of Scotch pine. Phytopathology 68:267-271.

18. Linzon, S.N. 1960. The development of foliar symptoms and the possible cause and origin of white pine needle blight. Can. J. Bot. 38:153-161.

19. Linzon, S.N. 1964. Studies on the nature and etiology of semimature-tissue needle blight on eastern white pine. PhD dissertation, Univ.Toronto, Toronto, Canada. 192 p.

20. Linzon, S.N. 1967. Histological studies of symptoms in semimature-tissue needle blight of eastern white pine. Can. J. Bot. 45:133-143.

21. Linzon, S.N. 1967. Ozone damage and semimature-tissue needle blight of eastern white pine. Can. J. Bot. 45:2047-2061.

22. Minter, D. W. 1981. *Lophodermium* on pines. Commonwealth Mycological Inst. Mycol. Paper. 147:1-54.

23. Stahl, S.S., Rogers, J.D., Adams, M.J. 1988. Observations on *Hendersonia pinicola* and the needle blight of *Pinus contorta*. Mycotaxon 31:323-337.

24. Stambaugh, W.J. 1952. A study of the needle cast diseases of conifers in Pennsylvania. J. For. 50:944.

25. Stephan, B.R. 1973. Untersuchungen zur Variabilitat von Loph*odermium pinastri*. I. Kulturvariaten. Eur. J. For. Path. 3:6-12.

26. U.S. Dept. Agr. 1960. Index of Plant Diseases in the United States. U.S.D.A. Agric. Handb. No. 165. 531 p.

Occurrence of *Tiarosporella Parca* in Switzerland: A Cause of Needle Blight?[1]

Ursula Heiniger and Manuela Schmid[2]

Abstract.—*Tiarosporella parca* occurs in all regions of Switzerland, from the lowlands to the subalpine region. Fruiting bodies are formed on red-brown needles on the tree during winter. They are also found in the litter. The possible role of *T. parca* in needle reddening and pathogenesis is discussed.

Introduction

Tiarosporella parca (Berk. & Br.) Whitney was described as *Sphaeropsis parca* Berk. & Br. in 1850 (1). In 1975 the fungus was placed in the genus *Tiarosporella* (14). It was found on needles of *Picea abies* (L.) Karst. from the UK and the CSSR and on *P. engelmanni* Parry and *P. glauca* (Moench) Voss from Canada. It was suggested that *Tiarosporella* species might cause needle blight because of "their frequent association with damaged areas of still living needles" (14). In 1984 it was isolated from brown needles in the southern FRG (9).

During a research project on needle diseases of *P. abies*, we noticed *T. parca* for the first time in the forest adjacent to our institute in February 1984. In the same year we also found it on 8 of 11 permanent plots in the Canton of Zuerich (5). Since then the fungus has been reported in Austria (4) and Norway (12). The fungus also has been isolated from green needles in Switzerland (6, 11). Its role has not yet been elucidated. It may be an endophyte that forms fruiting bodies on dying needles or it may be a pathogen causing needle reddening. It is also an open question why this fungus recently has been reported from several locations.

In this paper we will describe the symptoms associated with the occurrence of *T. parca* and some preliminary results regarding its biology. Its distribution in Switzerland is shown and compared to the distribution of *Lophodermium piceae* (Fuckel) Hoehn, sometimes associated with needle reddening. The possible role of *T. parca* will be discussed.

Methods

Between 1984 and 1988, branches and needle samples of *P. abies* from 99 sites were inspected. They were collected from permanent plots of the Canton Zuerich (5), from permanent plots of our institute and during field trips, or they were received from the local Forest Service for diagnosis. In July and August 1988 litter samples of *P. abies* were collected on 126 sites of the forest damage inventory in an 8 by 8 km grid over Switzerland. The needles were inspected with a dissecting microscope. The fungi were identified by their fruiting bodies. *Lophodermium piceae*, often exhibiting only anamorphs, was recognized by the typical black zone lines. Occasionally, and in doubtful cases, needles were dissected and the fungal fruiting body examined with the microscope. The occurrence of *T. parca, L. piceae*, and *Rhizosphaera* species was recorded. Other needle fungi, like the common *Chrysomyxa abietis* Unger and *C. rhododendri* (DC.) de Bary and occasional fungi belonging to the litter flora, were not considered. To detect *T. parca* in needle tissues, thin sections were stained with 0.01% aniline blue in 1/15 M phosphate buffer at pH 10 and inspected with an epifluorescence microscope.

Results

Association of needle reddening with Tiarosporella parca. — In recent years, yellowing followed by reddening of Norway spruce needles was observed beginning at the end of October (6). Old needles were affected but often all of the needles of secondary branches in the inner crown turned red-brown. First, the base of the needle turned yellow, then brown; slowly the whole needle lost its green colour. During this process *T. parca* fruiting bodies developed on the red-brown bases of needles which still had green tips (fig. 1).

[1] Paper presented at the I.U.F.R.O. W. P. S2.06.04 Foliage Disease Conference, 28 May-2 June, 1989, Carlisle, Pennsylvania.

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Figure 1.—Needle collected on October 28, 1989, with a green tip and a red-brown base exhibiting fruiting bodies of *Tiarosporella parca.*

During November and December most of the needles were shed (5). After needle cast, many thin twigs, devoid of needles, could be observed, giving the tree the "lametta" aspect.

Since 1984 we have inspected many branches with red-brown needles. Very often these needles have been densely covered with fruiting bodies of *T. parca* or the fruiting bodies have developed abundantly in a humid chamber after 3 to 5 days. Besides *T. parca*, *Lophodermium piceae, R. kalkhoffii* Bubak, and very rarely *R. oudemansii* Maubl., also were found. *Rhizosphaera* sp. occurred mainly on very old needles (>6 years) or occasionally on red first-year needles probably weakened by winter frost. *Lophodermium piceae* was associated with 3- to to 4-year-old needles. Usually the red brown needles were mixed with still green needles on the same shoot.

Distribution of Tiarosporella parca and Lophodermium piceae in Switzerland. — Branches and needles from 99 sites were inspected. *Tiarosporella parca* was found at 35 sites, *L.piceae* at 27 sites (fig. 2), and both fungi at 19 sites. *Rhizosphaera* sp. was found at 42 sites (not shown).

Of litter samples from 126 sites, 33 contained *T. parca*, 123 *L. piceae* (fig. 3), and 31 both fungi. *Rhizosphaera* sp. was present at 45 sites (not shown). Figures 2 and 3 show that *T. parca* and *L. piceae* are distributed in all regions of Switzerland. Both fungi were found even south of the Alps. Table 1 shows the occurrence of the fungi at different elevations above sea level. The highest site with *T. parca* was 1859 m, with *L. piceae* and *Rhizosphaera* sp. 2130 m.

Figure 2.—Occurrence of a) Tiarosporella parca and b) *Lophodermium* piceae in branches and needle samples collected in Switzerland, 1984 - 1988. Graphic: PHOAS/SFIFR

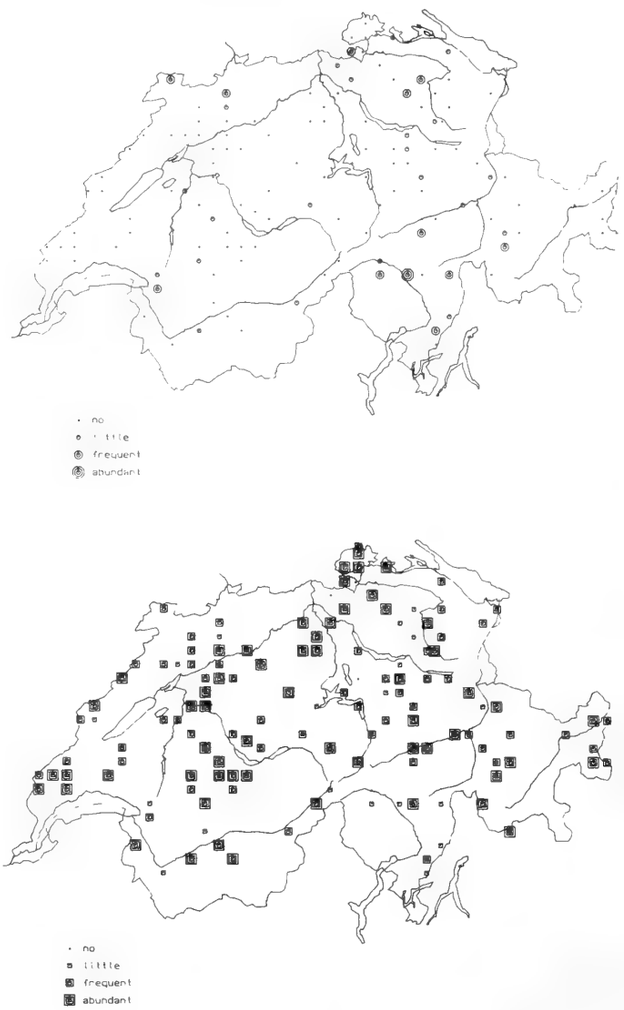

Figure 3.—Distribution of a) Tiaro*sporella parca* and b) *Lophodermium piceae* in litter samples collected in Switzerland, 1988. Graphic: PHOAS/SFIFR

Table 1.—Number (n) or % of sites with *Tiarosporella parca* or *Lophodermium piceae* per elevation range

	Elevation (meters above sea level)							
	<500		500-1000		1000-1500		>1500	
Sample	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Branches and needles								
N	27		44		21		8	
T. p.	14	52	28	64	9	43	3	38
L. p.	16	59	11	25	13	62	6	75
Litter								
N	9		49		45		23	
T. p.	2	20	9	18	12	27	6	26
L. p.	9	100	47	96	44	98	23	100

Biology of Tiarosporella parca. — *Tiarosporella parca* was isolated from green, living, symptomless needles of all ages. On needles turning yellow then red-brown in October, a great number of dark pycnidia were formed. At high humidity they opened up by a lateral slit. Mature pycnidia could be found beginning in November. Pycnidia were found in the litter throughout the year.

Tiarosporella parca grows well as a saprophyte. It can be easily grown on autoclaved spruce needles as well as on different agar media. In culture pycnidia form on autoclaved needles and on 1.2% malt agar. Sometimes the mycelium remains sterile. The optimum growth temperature was 15 C but growth was recorded between 5 and 25 C. The fungus survived freezing at -18 C.

The conidiospores germinated in water or in 100% humidity within 24 hours. The germ tubes grew in water and on the surface of water agar. In brown needles with *T. parca* fruiting bodies, fungal hyphae were restricted to the mesophyll.

Discussion

Distribution of Tiarosporella parca.— *Tiarosporella parca* usually is not mentioned in forest pathology text books, but it seems well known to mycologists who described it as early as 1850 (1). In 1975 the genus was revised and placed in the genus *Tiarosporella*. The type material came from Canada and Europe. In 1984 it was reported from the southern part of the FRG (9). In the same year we found this fungus for the first time at several locations in Switzerland, even south of the Alps. Since then it also has been reported in Austria (4) and Norway (12). It was, however, not found in investigations of needle fungi in Bavaria (7) and the FRG (3). It seems inconceivable that the fungus spread through Europe in only a few years. We believe that the fungus has always been present but not recognized because:

a) the reddening starts in October, whereas the fruiting bodies are produced during the winter season on needles still hanging on the tree.
b) the pycnidia disappear or are overgrown by other fungi in the litter.
c) the fungus seems not to be present in young trees. This may explain why many authors looking for endophytes did not isolate it (2, 13).
d) the fungus sporulates irregularly in culture.

The possible role of Tiarosporella parca in pathogenesis. — *Tiarosporella parca* grows well on dead needles and therefore is a good saprophyte. Comparison of figures 1 and 2 shows that at the sites where we collected litter, more *L. piceae* was present, whereas at the sites where we sampled fresh material more *T. parca* was present. This may indicate that *T. parca* is inferior to *L. piceae* in its survival in the litter. *Tiarosporella parca* has an endophytic stage (5, 11) and may only develop pycnidia on dying needles. The fact that the fruiting bodies appear on dying first-year needles or on stressed needles of recent grafts suggests at least that the fungus is weakly parasitic.

Biology. — In spite of an intensive search, we never have found *Darkera parca* Whitney, Reid & Pirozynski (14) and therefore cannot confirm the assumption that this is the teleomorph. In contrast to many needle fungi, *T. parca* produces viable pycnospores. The pycnidia open in late winter with humid conditions, but in spring spores are still present. An infection of the flushing needles in spring during rainy weather is therefore conceivable and supported by the fact that first-year needles may carry the fungus. The

infection of the branches in the upper crown of old Norway spruce may arise from fallen needles caught on branches. Not all Norway spruces on a specific site exhibit the reddening in fall with *T. parca* fruiting bodies on the needles. There may be genetic differences in the susceptibility of individual trees.

Site conditions. — We cannot link the sites with *T. parca* to climatic conditions. Sieber (11), however, found endophytic *T. parca* primarily at sites with low precipitation. Also at the site in Norway precipitation is low (12). No dependence on elevation has been found. There also seems to be no correlation with air pollution and forest damage. The most polluted areas, as derived from heavy metal analyses of spruce needles, are in the midlands and the north and northwest of Switzerland (8). *Tiarosporella parca* , however, is found in all regions.

The occurrence of *T. parca* in the litter cannot be correlated with the estimated needle loss at the collection sites (10). This fact corroborates the findings by Sieber (11) who isolated *T. parca* from damaged as well as from healthy trees. The Norwegian site where *T. parca* was found, however, is close to an industrial site with nickel and sulphur dioxide emissions (12).

Acknowledgement

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Literature Cited

1. Berkeley, M. J., Broome, C. E. 1850. Notices of British fungi. Ann. Mag. Hist. Ser. 2:365-380.

2. Butin, H. 1986. Endophytische Pilze in gruenen Nadeln der Fichte (*Picea abies* Karst.) Z. Mykol. 52:335-345.

3. Butin, H., Wagner, C. 1985. Mykologische Untersuchungen zur "Nadelroete" der Fichte. Forstw. Cbl. 104:178-186.

4. Cech, T., Tomiczek, C. 1988. *Tiarosporella parca* (Berk. and Br.) Whitney - erster Nachweis in Oesterreich. Eur. J. For. Path. 18:382-384.

5. Heiniger, U., Schmid, M. 1986. Nadelfall der Fichte. Untersuchungen zum jahreszeitlichen Verlauf des Nadelfalls und zum Vorkommen von Schuettepilzen im Kanton Zuerich. Schweiz. Z. Forstwes. 137:157-162.

6. Heiniger, U., Schmid, M. 1989. Association of *Tiarosporella parca* with needle reddening and needle cast in Norway spruce. Eur. J. For. Path.. 19:144-150.

7. Kowalsky, T., Lang, K. J. 1984. Die Pilzflora von Nadeln, Trieben und Aesten unterschiedlich alter Fichten (*Picea abies* (L.) Karst.) mit besonderer Beruecksichtigung vom Fichtensterben betroffener Altbaeume. Forstw. Cbl. 103:349-360.

8. Landolt, W., Guecheva, M., Bucher, J. B. 1989. The spatial distribution of different elements in and on the foliage of Norway spruce growing in Switzerland. Envir. Pollut. 56:155-167.

9. Rack, K., Butin, H. 1984. Experimenteller Nachweis nadelbewohnender Pilze bei Koniferen: I. Fichte (*Picea abies*). Eur. J. For. Path. 14:302-310.

10. Sanasilva - Waldschadenbericht. 1988. Eds. Bundesamt fuer Forstwesen und Landschaftsschutz, Bern and Eidg. Anstalt fuer das forstliche Versuchswesen, Birmensdorf, Switzerland. 47 p.

11. Sieber, T. 1988. Endophytische Pilze in Nadeln von gesunden und geschaedigten Fichten (*Picea abies* (L.) Karsten). Eur. J. For. Path. 18:321-342.

12. Solheim, H. 1989. Fungi on spruce needles in Norway. II. Notes about *Tiarosporella parca.* Eur. J. For. Path. (in press).

13. Suske, J., Acker, G. 1987. Internal hypae in young symptomless needles of *Picea abies*: electron microscopic and cultural investigation. Can. J. Bot. 65:2098-2103.

14. Whitney, H. S., Reid, J., Pirozynsky, K. A. 1975. Some new fungi associated with needle blight of conifers. Can. J. Bot. 53:3051-3063.

Current Status of Slash and Loblolly Pine Needlecast Diseases in the Gulf States[1]

G. A. Snow[2]

Abstract.—A needlecast disease of slash and loblolly pines (*Pinus elliottii* and *P. taeda*) that has recurred in south Mississippi each year since 1970 appears to be intensifying. Freezing weather is considered to be important for symptom expression because the disease has appeared each year immediately after the first killing frost or first period of freezing temperatures for at least 18 years. Needlecast diseases are becoming a serious problem for Virginia pine in Christmas tree plantings in Mississippi and Louisiana.

A severe needlecast disease was observed in early December 1970 on slash and loblolly pines (*Pinus elliottii* Engelm. and *P. taeda* L.) in southern Mississippi. Almost all trees in the three southernmost counties were affected. Foresters from several other southern states reported similar observations, and an aerial survey was made of the southern pine region in January 1971 (4). At this time, 53,852,440 acres were found to be affected.

Slash pines were affected more than loblolly pines, and longleaf pine (*P. palustris* Mill.) appeared to be immune. The disease was most severe on large trees. Although trees were affected uniformly through their crowns, there was much variation in the amount of needle damage among trees. On some trees only the needle tips were damaged, while on others, over two-thirds of the needle area was brown. Needles that had been formed in both 1969 and 1970 were affected. The distal portions of the needles were reddish brown, with a dark brown demarcation between the dead areas and the green bases of the needles. The appearance of these needles did not change any further until March and April 1971, when they became completely brown and fell from the trees.

Needles were collected from all states where the blight was observed (2). The most prevalent fungi identified on the needles were *Lophodermella cerina* Darker, *Ploioderma lethale* (Dearn.) Darker, and *P. hedgcockii* (Dearn.) Darker. These fungi were thought to have been the cause the disease, but the reason for the widespread epidemic was not determined.

Boyce (1) described a similar needle disease caused by *P. lethale*, which occurred from 1949 to 1952 on hard pines in the Atlantic States. The symptoms he described, however, consistently appeared in March and April—3 to 4 months later than those observed in the southern states in 1970. Whether this difference in the timing of symptom expression was due to differences in the fungi involved or to conditions under which the disease developed is unknown.

There has been a recurrence of needlecast each year since the 1970 observation, but the time that symptoms appear varies from December to April in southern Mississippi (3). The magnitude of the disease has not equaled the 1970-71 outbreak, but recent observations indicate that the blight may be intensifying. In late winter of 1988, 50 pairs of slash pine trees were chosen and marked on the Harrison Experimental Forest. One tree in each pair had the blight on all mature needles while the needles were healthy on the other tree. One year later, all previously infected trees exhibited a recurrence of the blight while four of the previously nonaffected trees developed disease symptoms. The disease appears to be clonal, and the number of trees affected in the area sampled increased from 1988 to 1989.

There appears to be a correlation between climatic conditions and the expression of symptoms, but the relationship is not well defined. Weather conditions during the spring and summer of 1970 were considered to be normal along the Mississippi coast, except that precipitation in April was below average. Autumn temperatures were unusual; those in November were well below normal, and a freeze (-6 C) on November 25 and 26 was followed by several periods of dense fog, each lasting from 4 to 7 days. The freezing weather is now thought to have been an important factor in the 1970 epidemic because every year since 1970, the disease symptoms have appeared within 2 to 3 days after the first killing frost or the first period of freezing weather.

[1] Paper presented at the I.U.F.R.O. W.P. S2.06.04 Foliage Disease Conference, 29 May - 2 June, 1989, Carlisle, Pennsylvania.

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Air pollution may be an important factor contributing to the disease. In 1970-71, a zone of heavy blight damage extended from Slidell, Louisiana, across southern Mississippi to north of Mobile, Alabama, down wind from the metropolitan areas of New Orleans and the Gulf Coast. An oil well located near the mouth of the Mississippi River in the Gulf of Mexico caught fire during the latter part of November and burned until early December 1970. The temperature inversions associated with the dense fogs during this time would have concentrated and held stationary whatever air pollutants were present.

One very severe episode of needle browning in loblolly pine needles was documented to be caused by chlorine gas that escaped from wrecked rail cars near Collins, Mississippi, in 1986. The pines and many other plants within a 30-square-mile area were damaged. A species of *Lophodermium* was prevalent on all affected needles 6 months after they were killed by the gas. Thus, the presence of fungi on brown needles does not prove that they are the primary cause of death; in this case the fungi were either secondary invaders or leaf inhabitors (endophytes), not pathogens.

Growing Virginia pine (*P. virginiana* Mill.) Christmas trees is a relatively new industry in the South. This industry is increasing rapidly in Mississippi and Louisiana; there are at least 250 growers in each state. Needle diseases are becoming a serious problem on these trees but little is known about the causal agents; this makes control difficult and expensive. Research to determine the cause of these disorders has begun at the Forestry Sciences Laboratory at Gulfport, Mississippi.

Literature Cited

1. Boyce, J. S., Jr. 1954. Hypoderma needle blight of southern pines. J. For. 52:496-498.

2. Czabator, F. J., Staley, J. M., Snow, G. A. 1971. Extensive southern pine needle blight during 1970-71, and associated fungi. Plant Dis. Rep. 55:764-766.

3. Snow, G. A. 1986. A needle blight of slash and loblolly pines in south Mississippi. p. 20-21. In: G.W. Peterson, (ed.). Recent Research on Conifer Needle Diseases. USDA For. Serv. Gen. Tech. Rep. WO-50. 106 p.

4. Wolfe, R. D., Drake, L. E., Peacher, P. H., Wilmore, D. H. 1971. A survey of pine needle blight damage in the South. USDA For. Serv. Rep. 72-2-3, 6 p.

Pathology of Japanese Cedar Twig Blight[1]

T. Kubono[2]

Abstract.—Through isolation and inoculation experiments a coelomycetous fungus was shown to be the cause of Japanese cedar twig blight. Field observations indicate that the fungus infects through twig buds and male flowers after being wounded by insects or frost. Mycelial mats of the fungus play an important role in lesion development. Symptoms and signs of the disease and morphology of the causal fungus are described.

Introduction

Twig blight of Japanese cedar, *Cryptomeria japonica* D. Don. (Kokuten-edagarebyo), is widespread throughout Japan and has been known as one of the common and important diseases in cedar plantations. This disease occurs on various aged trees; in particular, trees about 20 years of age are seriously damaged. When the cambial zones of the lateral branches are girdled by this pathogen, the branches are quickly killed and change to brownish red from autumn to early spring. Japanese cedar does not die of this disease, but dead branches cause a loss of growth in the young plantations.

The signs of this disease are characterized by white mycelial mats and minute, black acervuli on the diseased twigs (3, 4). However, germination of the conidium-like structures in the acervuli has not been observed, and other spore stages for transmission have not been detected. Therefore, the method of transmission is not yet clear.

As a result of isolation and inoculation experiments, this report demonstrates the pathogenicity of the coelomycetous fungus on the diseased twigs. Also, as a result of observations in diseased stands, the point of entrance of this pathogen has been confirmed.

Observation of the Disease in Plantations

Symptoms and signs of the disease.—Detailed observations were conducted from April, 1988 to March 1989 in 10-year-old and 20-year-old plantations located in a privately-owned forest in Shizukuishi-cho, Iwate Prefecture.

The first symptom appears as necrosis of the twig buds (fig. 1) and of the end of the twigs bearing male flowers in early spring (figs. 2 and 3). When the necrosis reaches the base of the twig, and girdles the branch cambium (fig. 4), the parts distal from the necrotic area die and change to a brownish-red from April to May. The dead brownish red branches are a typical symptom of this disease.

Minute, black acervuli are formed on the diseased twigs about the end of October (fig. 5). The white mycelial mats are another sign (fig. 6). They appear on the lesions from the end of March to the end of June. During the summer season, the mycelial mats on the green twigs disappear. Just after the mycelial mats disappear, the color of the green twig where the mycelial mats existed changes quickly to a brownish red (fig. 7).

Development of mycelial mats.—It was postulated that the mycelial mats are strongly related to lesion development. Therefore, the behavior of the mycelial mats was investigated. Twigs with white mycelial mats, 15 from a 10-year-old plantation and 13 from a the 20-year-old plantation, were selected and the edges of the mycelial mats were marked with a black pen.

The growth of the mycelial mats was measured four times from May to July. The results are shown in tables 1 and 2. Mycelial mats continued growing from May to the middle of June. They stopped growing about the end of June. They began to disappear in early July. All mycelial mats on the twigs disappeared by 20 July. Immediately after the mycelial mats disappeared during the summer season, discoloration and browning of the green parts occurred on all twigs to which mycelial mats had adhered. Furthermore, the severely affected branches developed cankers. Therefore, it seemed that the white mycelial mats creeping along the surface of the twigs had a strong relation to the development of the lesions.

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Isolation of Fungi From Diseased Parts and Mycelial Mats

Materials for fungi isolation were collected from two places. The isolation experiments were carried out from May to June, 1988, using cankered portions, mycelial mats and necrotic needles which developed immediately after the disappearance of the mycelial mats. Isolation pieces about 3 X 3 X 1 mm were sterilized with 70% ethyl alcohol and a 1:1000 solution of mercuric chloride, washed in two changes of sterilized water, and then plated on PDA. The plates were maintained at 7 C for about 1 month in the dark and then put on a laboratory desk under natural light.

The results of the isolation experiments are shown in tables 3 and 4. Depending on the samples, fungal genera as well as the percentage of the isolated fungi were different. But on the whole, a coelomycetous fungus, *Pestalotiopsis* spp. and *Fusarium* spp. were isolated from the diseased parts at a rate of more than 10%. It is remarkable that a coelomycetous fungus could be isolated at such a high rate from the diseased parts. Furthermore, this coelomycetous fungus was isolated from over 70% of the necrotic parts which developed immediately after the disappearance of the mycelial mats. The same fungus was isolated at a high rate from the mycelial mats, and also from the dead twig buds and dead male flowers on which the first symptoms were noted. The morphology of the conidial structures produced in culture was identical with that of the coelomycetous fungus on diseased cedar trees.

Inoculation

Inoculation of twig buds.—In April 1988, 8-year-old Japanese cedar trees were inoculated in the nursery. Ninety-four twig buds were chosen from five trees. Inocula were prepared by the Zinno method (15). A coelomycetous fungus colony which was cultured on PDA slants was divided in 2 to 3 mm blocks, and the blocks were transferred to a liquid medium in a shaking flask. After incubating for 10 to 15 days at 25 C, fungal blocks developed into sclerotium-like bodies 4-5 mm in diameter (fig. 8). These sclerotium-like bodies were used for inocula. Twenty-seven twig buds were inoculated without wounding by putting the inocula on the buds. Twenty-seven other twig buds served as controls by putting 1% PDA on the unwounded buds. Twenty twig buds were inoculated by putting inocula on buds wounded with a sterilized scalpel. Twenty other twig buds served as controls by putting 1% PDA on buds wounded with a sterilized scalpel. All twig buds were covered with clear plastic tape after inoculation. The tape was removed after 7 days. The affected twigs were observed for 6 months.

The results of the inoculation experiments with sclerotium-like bodies are shown in table 5. All wounded twig buds and 7 % of the non-wounded twig buds were infected (fig. 9). However, no controls showed symptoms in either of the trials. Necrotic parts with mycelial mats developed on the inoculated buds of non-wounded twig buds 2 months after inoculation. Meanwhile, necrotic parts developed on the wounded twig buds 1 month after inoculation. At this time, mycelial mats had already appeared on the necrotic parts. These initial symptoms developed into the typical twig blight disease.

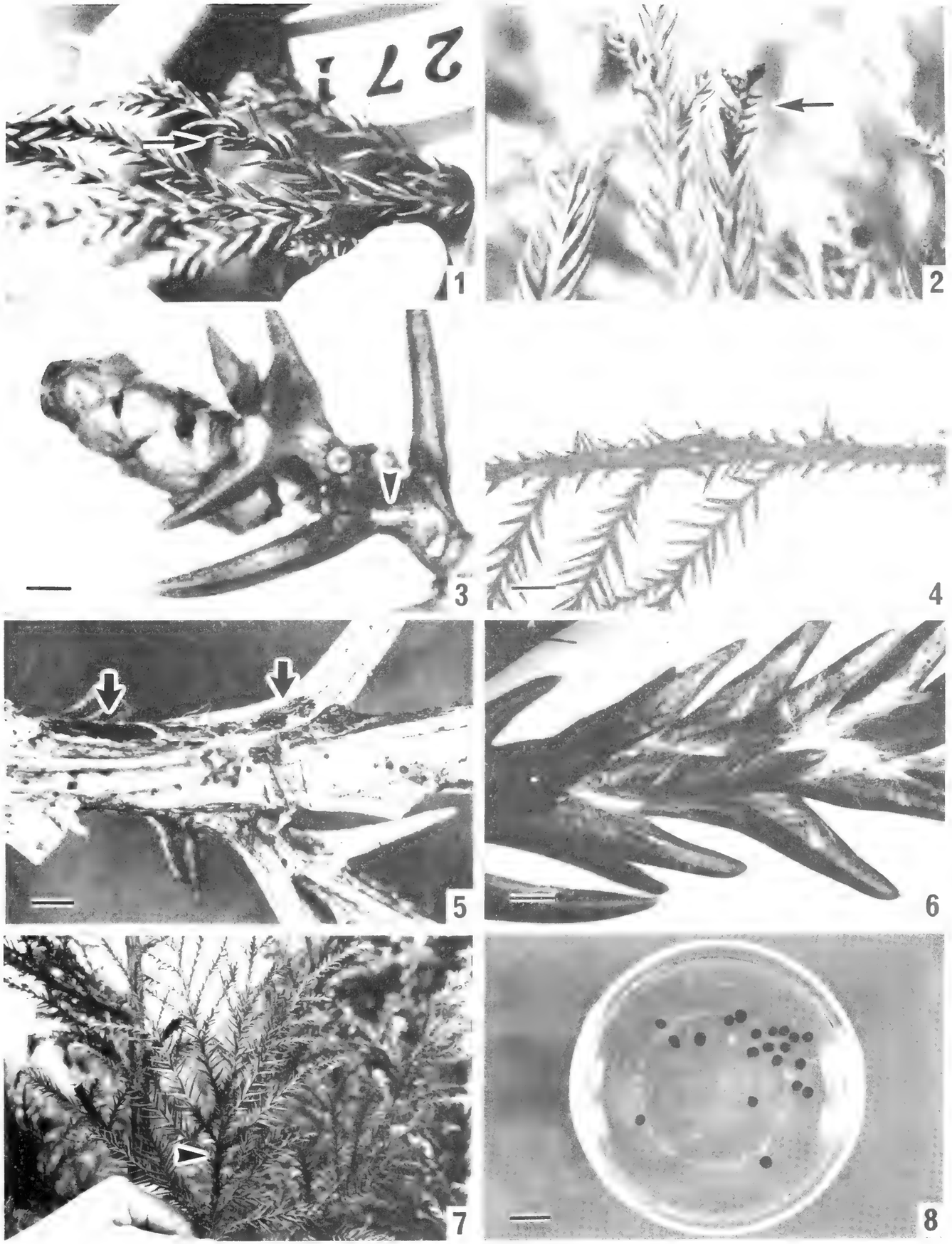
Inoculation of branches.—Twenty branches on five trees were chosen. Green parts of the lateral branches were wounded with a 1 mm diam. needle at two points per branch. Sclerotium-like bodies used as inocula were inserted into one of the 1 mm diam. holes at the central part of a lateral branch. The other hole was used as the control. The inoculated holes were covered with clear plastic tape which was removed after 7 days. The length of the necrotic lesions was measured over a 6 month period.

Results of these inoculations are shown in table 6. All inoculated parts were infected and lesions formed. About 1 month after inoculation, the lesion lengths ranged from 8 to 27 mm, the average being 16.0 mm. Mycelial mats appeared on seven of the 19 lesions. Two of the 19 lesions developed into cankers (fig. 10). About 6 months after inoculation, the length of these lesions ranged from 17 to 70 mm, with an average of 37.5 mm. Though some mycelial mats were observed on one of the 19 lesions, all other mycelial mats had already disappeared. Also four of the 19 lesions developed into cankers. Many black stromata developed on two of the lesions, but these stromata did not develop into fruiting bodies. One of the inoculated branches had already died back by being girdled by necrosis at the cambial zone. The holes of the 16 controls had healed over completely.

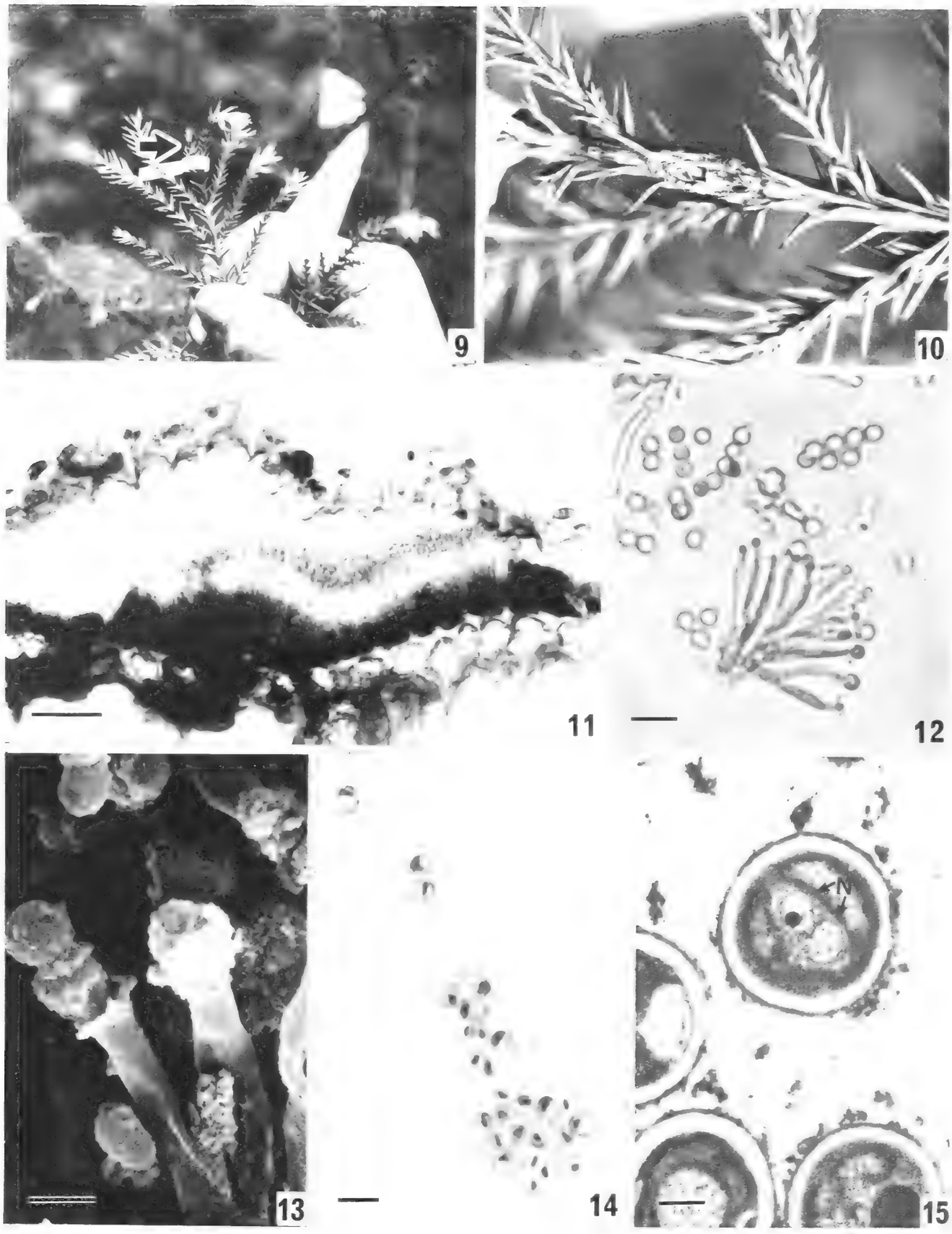
Morphological Characteristics of the Fungus

Spore-like bodies formed on dead twigs, sterilized twigs and on PDA slants. The fungus was observed with a light microscope, a scanning electron microscope and a transmission electron microscope.

Acervuli were immersed, intraepidermal, erumpent, and black. Basal stromata were pseudoparenchymatous, composed of dark brown to black cells (fig. 11). Conidiogenous cells were enteroblastic, cylindrical, hyaline, smooth walled (fig. 12). Conidia were globose to obovoid, unicellular, pale brown, truncate at the base, 3.0-4.0 X 2.0-3.0 µm, with rugose wall surfaces, and produced in chains from the acervuli (fig. 13).



Figures 1-8.—Twig blight of Japanese cedar. 1. Initial symptom on a blighted needle bud (arrow). 2. Initial symptom on a blighted twig end bearing male flowers (arrow). 3. Mycelial mats (arrow) at the base of a dead male flower. 4. A typical cankered branch. 5. Acervuli (arrows). 6. Mycelial mats creeping along the surface of a twig. 7. Browning (arrow) of a branch found after mycelial mats disappeared during summer season. 8. Sclerotium-like bodies produced in flask in shake culture. (Scales: 3=1 mm; 4=1 cm; 5=1 mm; 6=1 mm; 8=5 mm).



Figures 9-15.—Twig blight of Japanese cedar. 9. Dead twigs (arrow) resulting from inoculation of wounds. 10. A canker formed on a branch following inoculation. 11. A cross section of an acervulus of the twig blight fungus. 12. Conidia produced in chains from conidiogenous cells in an acervulus. 13. A scanning electron micrograph of conidia and conidiogenous cells. 14. Conidia with two nuclei, stained with HCl-Giemsa. 15. A transmission electron micrograph of conidia with two nuclei (N). (Scales: 10=1 cm; 11=5 μm; 12=10 μm, 13=3 μm, 14=5 μm; 15-1 μm).

Results of HCl-Giemsa staining (fig. 14) and TEM (fig. 15) showed that many of the conidia had two nuclei. However, germination of the conidia has not been obtained. The morphological characteristics of this fungus does not fit into hitherto known genera of the Coelomycetes.

Discussion

A specific coelomycetous fungus was isolated at a high rate from the diseased parts and from the white mycelial mats creeping on the surfaces of twigs. The morphological characteristics of the conidial structures in culture were identical to those produced on the host. As a result of the inoculation of 8-year-old Japanese cedars, pathogenicity of the fungus was proven. Furthermore, the lesions developed into the typical symptoms of this disease. Mycelial mats appeared from the lesion formed by inoculation. Consequently, it was determined that the twig blight pathogen of Japanese cedar is the coelomycetous fungus found on the diseased parts. Furthermore, the enlargement of the lesions is closely related to the behavior of the mycelial mats.

The behavior and characteristics of the white mycelial mats is similar to that of thread blight caused by *Ceratobasidium anceps* (Bres. et Syd) Jackson. In Japan, thread blight is a common and important disease of *Ginkgo biloba* Linn (5, 6). The white rhizomorphs of *Ceratobasidium anceps* extend to the petioles along the under side of the twigs. White mycelial mats of the twig blight fungus develop on the under surfaces of the branches and sometimes girdle the branches. They are white at first but later become brown. The mycelial mats are most active from early spring to the rainy season. The white active mycelial mats continue to grow. With a rise in temperature in summer, the mycelial mats seem to enter the host tissue. It is suspected that this is why the mycelial mats disappear from the twig's surface in summer. However, their role and behavior remain unclear and more investigation is necessary.

As a result of observations in the plantations, necrosis, which seemed to be the first symptom, was found on the twig buds. The results of isolations and inoculations suggest that the twig buds are the sites for the onset of this disease. Inoculations of both wounded and nonwounded twig buds resulted in infection. Inoculation of wounds resulted in 100 percent infection. It seems that the occurrence of fungus in the twig buds is strongly related to wounds. In the field, twig bud necrosis caused by late frost damage is sometimes observed. It is thought that Japanese cedar buds are susceptible to damage by late frosts (12).

Botrytis cinerea Persoon is thought to invade the host tissues through the dead buds of the Todo fir resulting from frost (2). *Scleroderis* canker of the Todo fir is also strongly associated with snow and cold damage (13,14).

Japanese cedar twigs are occasionally wounded by insects such as *Argyrestihia anthocephala* Meyrick (8) and *Contarinia inouyei* Mani (9). *Hypoxylon mammatum* (Wahl.) Miller (1), *Endothia parasitica* (Murrill) P.J. et H.W. Anderson (7) and *Nectria coccinea* (Persoon ex Fries) Fries. (10) are thought to invade their host trees through tissues injured by various insects. To now, it has been thought that twig buds wounded by insects did not become an invasion point of the fungus in Japanese cedar plantations. However, in early spring white mycelial mats have been observed creeping on the surfaces of twig buds killed by *Contarinia inouyei*. Thus this pathogen may be associated with *Contarinia inouyei*. Consequently, with regard to frost damage and insect damage of twig buds, it is necessary to study more precisely the transmission of this pathogen after germination of the conidium-like structures has been accomplished. However, we can assume the following from the results of the present study. First, conidium-like structures of the pathogen reach twig buds wounded by insects or frost. After that, mycelial mats develop on the twig, resulting in twig blight. The mycelial mats extend over the surface of the green parts from the dead twigs to the primary branches through the second branch. Finally, dieback of the primary branch results due to the effects of the mycelial mats.

The causal fungus has distinctive morphological characteristics. According to Sutton (11), this fungus belongs to "phialostromatineae". However, the morphological characteristics of this fungus do not fit those of hitherto known genera of the Coelomycetes. The scientific name of this pathogen will be determined after more precise taxonomical studies.

Literature Cited

1. French, D. W., Oshima, N. 1959. Host bark characteristics and infection by *Hypoxylon pruninatum* (Klot.) Cke. Forest Sci. 5:255-258.

2. Imai, S. 1948. [Buds blight of Todo-fir seedlings.] Ann. Phytopath. Soc. Japan 13:58. (In Japanese).

3. Ito, K. 1954. [Dieback of Japanese cedar.] Forest Pests 24:239-240. (In Japanese).

4. Ito, K. 1965. [*Chloroscypha* needle blight and twig blight of Japanese cedar.] Forest Pests 14:38-40. (In Japanese).

5. Ito, T. 1957. [Thread blight caused by *Pellicularia koleroga* Cooke.] J. Jpn. For. Soc. 39:483-485. (In Japanese).

6. Ito, T. 1958. [Thread blight of *Ginkgo biloba* Linn. caused by *Pellicularia koleroga* Cooke.] Bull. For. & For. Prod. Res. Inst. 105:11-18. (In Japanese).

7. Kato, K. L. 1963. [Investigation on the occurrence of chestnut blight in Kanagawa prefecture.] p. 37-70. In: Investigation on occurrence of chestnut blight. G. Nishikado (ed.). Agr. For. and Fish. Res. Council Secretar., Tokyo. (In Japanese).

8. Kato, Y. 1956. [A few insects that injure the needles of Japanese cedar.] Forest Pests 5:257-260. (In Japanese).

9. Kimura, S., Yanbe, T., Igarashi, M. 1962. [Study on the ecology of Con*tarinia inouyei* Mani.] Trans. Meet. Jap. For. Soc. Tohoku Branch 13:117-124. (In Japanese).

10. Shigo, A. L. 1963. Beech bark disease. U.S. Dept. Agric. Forest Pest Leaflet 75. 8 p.

11. Sutton, B. C. 1980. The Coelomycetes. Commonwealth Mycological Inst., Kew, England. 696 p.

12. Tokushige, Y. 1960. [Frost damage in the young stands of Japanese cedar.] Trans. Meet. Jap. For. Soc. Kyusyu Branch 14:128. (In Japanese).

13. Yokota, S. 1970. [Occurrence of *Scleroderris* canker of Todo-fir.] Forest pests 19:300-302. (In Japanese).

14. Yokota, S. 1971. [Scleroderris canker of Todo-fir.] Hoppo Ringyo 23:251-255. (In Japanese).

15. Zinno, Y. 1979. Studies on artificial sporulation of *Cercospora sequoiae* Ellis et Everhart, the needle blight fungus of *Cryptomeria japonica*. Bull. For. & For. Prod. Res. Inst. 302:1-77.

Table 1.—Growth and disappearance of mycelial mats on twigs of a 10-year-old stand

Sample No.	Size of Mycelial Mats on Different Dates			July 20, 1987		
	June 2	June 25	July 10	Mycelial mats	Necrotic needles & twigs	Stromata
	mm	*mm*	*mm*			
1	95	100	*	*	+	+
2	21	*	*	*	+	-
3	32	*	*	*	+	+
4	76	76	*	*	+	+
5	68	87	97	*	+	+
6	17	*	*	*	+	+
7	27	30	32	*	+	+
8	53	*	*	*	+	-
9	35	*	*	*	+	+
10	23	28	28	*	+	+
11	30	*	*	*	+	+
12	24	47	*	*	+	-
13	72	*	*	*	+	+
14	17	*	*	*	+	+
15	36	44	44	*	+	-

* = Disappearance; + = present; - = absent.

Table 2.—Growth and disappearance of mycelial mats on twigs of a 20-year-old stand

Sample No.	Size of Mycelial Mats on Different Dates			July 20, 1987		
	May 29	June 25	July 10	Mycelial mats	Necrotic needles & twigs	Stromata
	mm	*mm*	*mm*			
1	40	68	74	*	+	-
2	46	70	60	*	+	-
3	24	54	67	*	+	-
4	38	*	*	*	+	-
5	26	40	25	*	+	-
6	36	40	*	*	+	+
7	84	84	90	*	+	+
8	66	70	60	*	+	+
9	20	28	*	*	+	-
10	13	39	46	*	+	-
11	31	31	*	*	+	-
12	51	51	76	*	+	-
13	20	40	63	*	+	-

* = Disappearance; + = present; - = absent.

Table 3.—Fungi isolated from the diseased parts in a 10-year-old stand

Fungi isolated	Cankered branches	Mycelial mats	Necrotic needles & twigs	Total
	%	%	%	%
A coelomycetous fungus	54	57	77	63
Pestalotiopsis sp.	16	23	15	18
Fusarium sp.	12	3		5
Phomopsis sp.	3			1
Cladosporium sp.	3	8		4
Phyllosticta sp.			3	1
Epicoccum sp.		3		1
Unidentified	12	6	6	7
Total number of fungi isolated	35	35	35	105
Total number of chips plated	35	35	35	105
Rate of isolates obtained (%)	100	100	100	100

Table 4.—Fungi isolated from the diseased parts in a 20-year-old stand

Fungi isolated	Cankered branches	Mycelial mats	Necrotic needles & twigs	Total
	%	%	%	%
A coelomycetous fungus	43	35	72	52
Pestalotiopsis sp.	9	41	28	26
Fusarium sp.	21	9		8
Phomopsis sp.	9			3
Cladosporium sp.	9	9		
Phoma sp.		3		1
Rhinocladiella sp.		3		1
Unidentified	9			3
Total number of fungi isolated	32	34	47	113
Total number of chips plated	35	35	35	105
Rate of isolates obtained (%)	92	97	134	107

Table 5.—Results of inoculation of twig buds with sclerotium-like bodies

Treatments	No. of twig buds inoculated	No. of twig buds infected			Total (%)
		June 10, 1988	July 1, 1988	Nov. 2, 1988	
No wound	27	0	0	2	7
No wound (control)	27	0	0	0	0
Wound *	20	0	20	20	100
Wound * (control)	20	0	0	0	0

* = wounded with a scalpel

Table 6.—Results of inoculation experiments with sclerotium-like bodies to the middle portions of lateral branches

Branch No.	Length of lesion			
	Inoculated Twigs			Controls
	10 May 1988	15 June 1988	4 Nov. 1988	4 Nov. 1988
	mm	*mm*	*mm*	*mm*
1	-	22	50	o
2	-	15	30	o
3	-	18	30	o
4	-	15	50	o
5	-	11	35	o
6	-	++	++	20
7	-	12 +M	35 +M,stroma	o
8	-	8	25 +canker	o
9	-	10	30	o
10	-	8	60 +stroma	o
11	-	18 +M	55	o
12	-	20	40	o
13	-	18 +M	40 +canker	o
14	-	27 +canker	50 +canker	o
15	-	18 +M	55	o
16	-	14 +M	20 *	o
17	-	20 +M	70 +canker	++
18	-	17 +M,canker	17	o
19	-	18	40	o

+canker = formation of canker; +stroma= formation of stroma;
+M = formation of white mycelial mats; * = killed by girdling;
++ = slight lesion; o = healed over

Variable Defoliation Levels in *Picea Pungens* By *Rhizosphaera Kalkhoffii* [1]

James A. Walla[2]

Abstract.—*Rhizosphaera kalkhoffii* often causes severe defoliation of *Picea pungens* in North Dakota, whereby only the one or two youngest needle complements remain green and attached to branches. More needle complements (up to eleven) remain green and attached to branches on some trees adjacent to severely defoliated trees. A near continuous gradation between the extremes of defoliation was noted among trees. The cause of the observed variable defoliation levels appears to be genetic resistance, which would be a valuable trait to incorporate into *P. pungens* seed orchards and cultivars.

Introduction

Needlecast, caused by *Rhizosphaera kalkhoffii* Bubak, is the most widespread damaging disease of *Picea pungens* Engelm. (Colorado blue spruce) in North Dakota (5). *Picea pungens* is among the most important tree species in the state, being utilized for protection, beautification and profit in rural and urban plantings. Damage from *R. kalkhoffii* consists of premature defoliation and branch death. In North Dakota, older needles are usually cast in a pattern consistent with that reported elsewhere in the United States (3). *Picea pungens* in disease free or lightly infected plantings hold up to 13 complements of green needles. In contrast, severely infected trees hold as few as one complement (current-year) of green needles. The most serious damage commonly occurs during the period following canopy contact (often ages 10 to 25 years). Tree mortality as reported in nearby states (4) has not been recorded in North Dakota but severe defoliation and branch death may result in tree removal.

Currently available control practices involve cultural and chemical methods. Because these control methods are not widely utilized, serious damage occurs often. A passive control method that prevents serious damage is needed. Resistance to *R. kalkhoffii* would provide such a method, but is not currently available.

In 1986, individuals of *P. pungens* in two North Dakota plantings were observed to have little defoliation due to *R. kalkhoffii* when compared to adjacent severely defoliated trees. This variability in defoliation appeared to be due to genetic resistance. Skilling and Nicholls (3) found some indications of genetic resistance to *R. kalkhoffii* in heavily infected *P. pungens* plantations in the Great Lakes region of the United States. A few trees were unaffected in most heavily infected plantations. No other reports of genetic resistance to *R. kalkhoffii* within any spruce species were found. However, resistance between spruce species (1) and within pine species (2) is known. This paper documents field observations concerning variability of *R. kalkhoffii* defoliation levels in plantings of *P. pungens* in North Dakota.

Materials and Methods

Visual identification of trees with reduced defoliation was possible only when adjacent trees had severe defoliation. Hence, heavily infected spruce plantings were observed for trees with reduced defoliation. Observations were made in northeast and north central North Dakota where previous surveys found *R. kalkhoffii* to be common and damaging (5). A total of 5,078 *P. pungens* in 20 plantings of various ages were examined from 8-11 June 1987. At that time, the 1987 needles were sufficiently developed to be considered the current-year complement.

Defoliation was assessed as the number of complements of green needles on branches on the lower crown of trees (ca. lower 2 m). Severely defoliated trees were considered to be those that held two or fewer complements of green needles. Trees were arbitrarily recorded as notably less defoliated if they held five or more complements of needles without

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disease symptoms and four or more complements of needles without pycnidia when adjacent trees were severely defoliated. On notably less defoliated trees, the number of complements of green needles and the presence of disease symptoms and signs on each retained complement of needles were recorded.

Results and Discussion

Severely defoliated trees were not found distributed randomly in plantings. Rather, they occurred in groups. Trees in some parts of some of the observed plantings were not severely defoliated. A tree that was severely defoliated was likely to be next to another severely defoliated tree. It appears that most of the *P. pungens* germplasm used in North Dakota is highly susceptible to defoliation by *R. kalkhoffii* because most trees in heavily infected areas were severely defoliated.

Scattered among the severely defoliated trees in heavily infected plantings were less defoliated trees. Gradations among trees in retention of green needles up to eleven complements were found on some trees in generally heavily infected plantings. No trees were found without disease symptoms or signs if nearby trees were severely defoliated. Eleven notably less defoliated trees were identified in 1987. Of those trees, three retained five green needle complements (pycnidia not seen on any of those needles), two retained six green needle complements (pycnidia seen on the sixth complement of one tree, pycnidia not seen on needles of the other tree), one retained seven green needle complements (pycnidia not seen on any of those needles), three retained eight green needle complements (pycnidia seen on the fifth through the eighth complement of two trees, pycnidia not seen on needles of the other tree), and two retained eleven green needle complements (pycnidia seen on the fourth through the eleventh complement of one tree and on the fifth through the eleventh complement of the other tree). Incidence of notably less defoliated trees was 0.2% (11 of 5,078) in the plantings observed in 1987. Error is most likely in this rate of incidence because some trees recorded as notably less defoliated may have escaped infection and because some trees that could have expressed reduced defoliation could not be detected because they occurred in areas of plantings with less heavy infections.

Some trees that retained several complements of green needles at crown heights where adjacent trees held only one complement (e.g. 2 m high) held fewer complements lower in the crown (e. g. 0.5 m high). It appeared that the factor causing less defoliation could sometimes be overcome under higher inoculum levels and more favorable environments for infection that presumably occur closer to the ground. On some trees which held several complements of green needles, a number of older complements of green needles were infected as indicated by the presence of *R. kalkhoffii* pycnidia. On those trees, the presence of older infected needles suggests either the unlikely recent simultaneous infection of a number of complements or a greater tolerance to infection whereby the needles remain green for a longer time after infection. Hypothetically, the needles might be infected at the same age as severely defoliated trees, but needles on less defoliated trees do not turn brown and cast until a later age. Research to determine what is happening in these needles in needed. In windbreak, wildlife, and ornamental plantings, this reduced defoliation would provide higher value trees. However, the trees would be expected to grow slower and be more stressed than trees with no infection.

The cause of the observed variable defoliation levels in *P. pungens* appears to be genetic resistance, likely similar to the situation noted by Skilling and Nicholls (3). If such is the case, most *P. pungens* germplasm in North Dakota has little resistance to *R. kalkhoffii*. There appears to be a near continuous gradation in this putative resistance among trees between the noted extremes of defoliation. Two factors (number of green needle complements without symptoms and those without pycnidia) were used in identifying putatively resistant trees. The two factors appear to vary independently except that green needles must be present before they can be found to have pycnidia.

A total of 16 putative highly resistant trees (trees with five or more green needle complements retained on branches and four or more green needle complements without pycnidia) were identified in 1986 and 1987. Evaluation of propagules from these trees is needed to determine if the putative disease resistance can be substantiated under controlled testing. If substantiated, heritability of the trait will be examined with the eventual goal of incorporating resistance to *R. kalkhoffii* into *P. pungens* seed orchards.

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Literature Cited

1. Kumi, J., Lang, K. J. 1979. The susceptibility of various spruce species to *Rhizosphaera kalkhoffii* and some cultural characteristics of the fungus *in vitro*. Eur. J. For. Path. 9:35-46.

2. Shibata, M. 1969. Breeding on *Rizoshaera* resistance to *Pinus densi-thunbergii*. Oji Institute for Forest Tree Improvement Technical Note No. 82. 1 p.

3. Skilling, D. D., Nicholls, T. H. 1974. Rhizosphaera needlecast. Am. Christmas Tree J. 18(3):21-23.

4. Skilling D. D., Waddell, C. D. 1975. Control of Rhizosphaera needlecast in blue spruce in Christmas tree plantations. Plant Dis. Rep. 59:841-843.

5. Walla, J. A., Stack, R. W., Nelson, D. R. 1987. Diseases and arthropod pests of spruce in North Dakota. (Abstr.) Phytopathlogy 77:1757.

Preliminary Information on the Life Cycle of *Lophodermium Piceae* in Norway Spruce[1,2]

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Abstract.—Studies on the life cycle of *Lophodermium piceae* indicate that the fungus can live as an endophyte in symtomless Norway spruce needles for several years and develops fruiting bodies only when the needles are dying due to senescence or stress (air pollution, drought, deficiency, damage by insects, etc.). Conidiomata with mature conidia can be found on dying but still attached needles in late summer or early autumn. Apotheca develop beginning in mid-December and mature beginning approximately mid-April of the following year. Ascospore discharge occurs until the end of August with a distinct maximum about the end of May and beginning of June. Evaluation of daily spore catches showed a close correlation between the amount of precipitation and ascospore discharge. Maximum ascospore availability coincides with the flushing of spruce shoots and needles, when infection occurs.

Introduction

In connection with forest decline, an increasing number of fungus species can be observed which until now have been defined as saprophytes or weak parasites. The biology of most of these fungi is still unknown and rarely investigated. The ascomycete *Lophodermium piceae* (Fuckel) v. Höhnel belongs to these common but little studied fungi. *Lophodermium piceae* occurs with other fungi in needles of Norway spruce (*Picea abies* (L.) Karst.). To gain a better knowledge of its biology, collections of *L. piceae* from many parts of its natural range in Europe were examined. For life cycle studies, additional samples were collected periodically for more than 2 years. Preliminary and summarized observations are given here. The results of the extensive investigations are part of a doctoral thesis (5) and will be published elsewhere in more detail.

Materials and Methods

The development of conidiomata and ascomata of *L. piceae* was studied in still-attached or in fallen needles of a 23-year-old Norway spruce stand in the Arboretum of the Institute of Forest Genetics at Grosshansdorf (Schleswig-Holstein; northern Germany) from 1986 to 1988. Samples were collected periodically at least every second week and examined microscopically in the laboratory. In addition, more than 800 needle samples with *L. piceae* from many parts of central and northern Europe also were examined.

Ascospore discharge was studied weekly in 1987 and daily in 1988 using microslide spore traps. During some periods, the daily temperature, air humidity and precipitation between and below the tree crowns were recorded simultaneously. In May and June 1988 the development of shoots and needle flushing also were evaluated.

Results

The results of the life cycle studies on *L. piceae* are summarized in figure 1 which shows an annual cycle divided into monthly parts (January to December) and the four main sectors of spring (F), summer (S), autumn (H), and winter (W).

During May and July the recently flushed current-year needles of Norway spruce are invaded by ascospores (fig. 1, I). The fungus lives in the symptomless needles as an endophyte for less than one year or

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longer (fig. 1, II), depending on the natural or artificial senescence of the needles. About the end of summer and the beginning of autumn the development of conidiomata can be observed on dying but still-attached needles. At first the conidiomata are visible as very small, concolorous vesicles in the needle epidermis. The conidia are produced inside, ripen until December and then are extruded (fig. 1, III). Then the conidiomata become overmature and turn dark brown or black. The initiation of ascomata formation can be found in December on needles in the tree crown or on fallen needles (fig. 1, IV). There is a rapid development of apothecia which can be seen macroscopically in early January. Paraphyses, and later asci and ascospores, develop within the apothecia (see drawings in fig. 1, IV). About mid-April the first mature ascospores can be found inside the asci. Then the apothecia open by a longitudinal slit and the ascospores are discharged (fig. 1, V). Ascospore discharge was measured in 1987 and 1988 and showed a pattern illustrated in figure 2. Ascospores were trapped from April to August with a distinct maximum in the first half of June. The rhythm of ascospore discharge was influenced mainly by the daily amount of precipitation. The number of ascospores collected on the spore trap was closely correlatd with the amount of precipitation (5). Temperature did not affect ascospore discharge and humidity affected it only indirectly. Ascospore discharge and the flushing of spruce shoots and needles coincides very well from May to June, allowing a new cycle of *L. piceae* to start. The life cycle of the fungus ends when the apothecia become overripe and when the apothecia and needles decompose in the litter (fig. 1, VI).

Discussion

Until now there has been very little information about the life cycle of the needle endophyte, *L. piceae*. This lack is filled, in part, by results of these studies, which are also in a good accordance with our own observations on material from other regions, although some slight changes can be caused by environmental and geographical conditions.

A comprehensive knowledge of the biology of a fungus is necessary for a better understanding of its role and importance within an ecosystem. The hitherto existing information about the life cycle of *L. piceae* was very scarce and fragmentary. There are only a few reports regarding the appearance, formation or maturation of conidiomata and ascomata (1, 2, 4, 8, 9, 10), and only a few of these agree with our own observations.

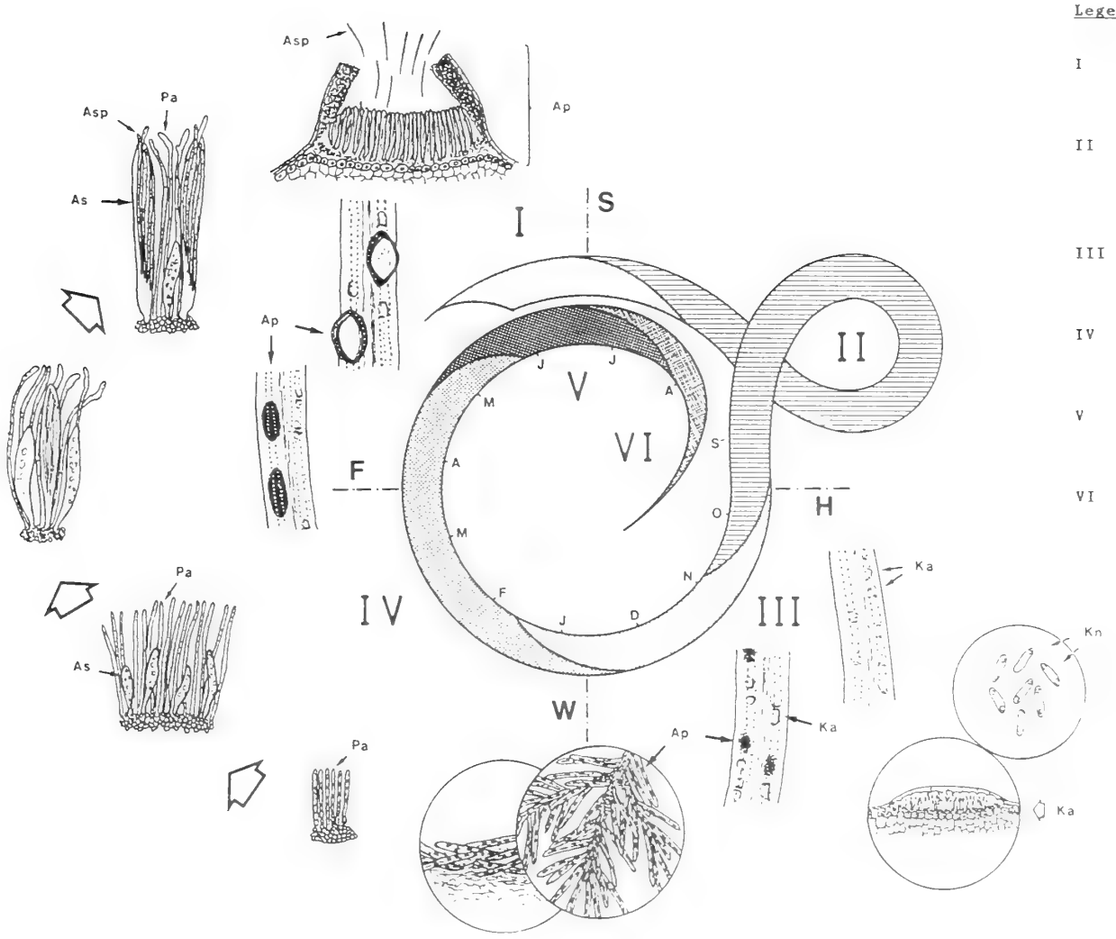
There is no information about ascospore discharge, but the close correlation between precipitation and sporulation is in good accordance with the results of Lanier and Aussenac (3) and Rack (6) concerning *L. pinastri* (Schrad.) Chev. The coincidence of ascospore discharge and flushing of the host also agrees with the behavior of *L. pinastri* and Scots pine (*Pinus sylvestris* L.) (7).

Although several questions of the life cycle of *L. piceae* were clarified, there are still points which should be investigated in more detail.

Literature Cited

1. Ferdinandsen, C., Jørgensen, C. A. 1938. Skovtraeernes Sygdomme.Gyldendalske Boghandel, Nordisk Forlag, Kobenhavn, 570 p.
2. Hilitzer, A. 1929. Monografická studie o ceskych druzích rádu Hysteriales a o sypavkách jimi pusobenych. Vedecké spisy vydávané. Ceskoslovenskou Akademii Zemedélskou 3. 161 p.
3. Lanier L., Aussenac, G. 1969. Contribution a l'étude du *Lophodermium pinastri* (Schrad.) Chev.: Résultats des captures de spores en 1967. Annales de Phytopathologie 1:449-472.
4. Lind, J. 1913. Danish fungi as represented in the herbarium of E. Rostrup. Gyldendalske Boghandel - Nordisk Forlag, Copenhagen. 648 p.
5. Osorio, M. 1989. Zur Biologie des in Fichtennadeln vorkommenden Pilzes *Lophodermium piceae* (Fuckel) v. Höhnel. Dissertation. Georg-August-Universität, Göttingen. 175 p.
6. Rack, K. 1963. Untersuchungen über die Kiefernschütte. II. Die Entwicklung der Fruchtkörper. Ztsch. f. Pflanzenkrankheiten 70:257-272.
7. Rack, K. 1963. Untersuchungen über die Kiefernschütte. III. Die Phänologie der Fruchtkörper und ihre epidemiologische Bedeutung. Ztsch. f. Pflanzenkrankheiten 70:385-398.
8. Rack, K., Butin, H. 1984. Experimenteller Nachweis nadelbewohnender Pilze bei Koniferen. I. Fichte (*Picea abies*). Eur. J. For. Path. 14:302-310.
9. Roll-Hansen, F. 1969. Soppsykdommer på Skog traer. Vollebekk. 173 p.
10. Rostrup, E. 1891. Undersøgelser over Snyltesvampes Angreb paa Skovtraeer i 1883-1888. Tidsskr. Skovbrug, 12:175-238.

Fig. 1. LIFE CYCLE OF *Lophodermium piceae* ON NORWAY SPRUCE



Legend:

I = inoculation of current year needles

II = endophytism for several years, exceptionally for one year

III = formation, development and maturity of conidiomata

IV = formation and development of apothecia

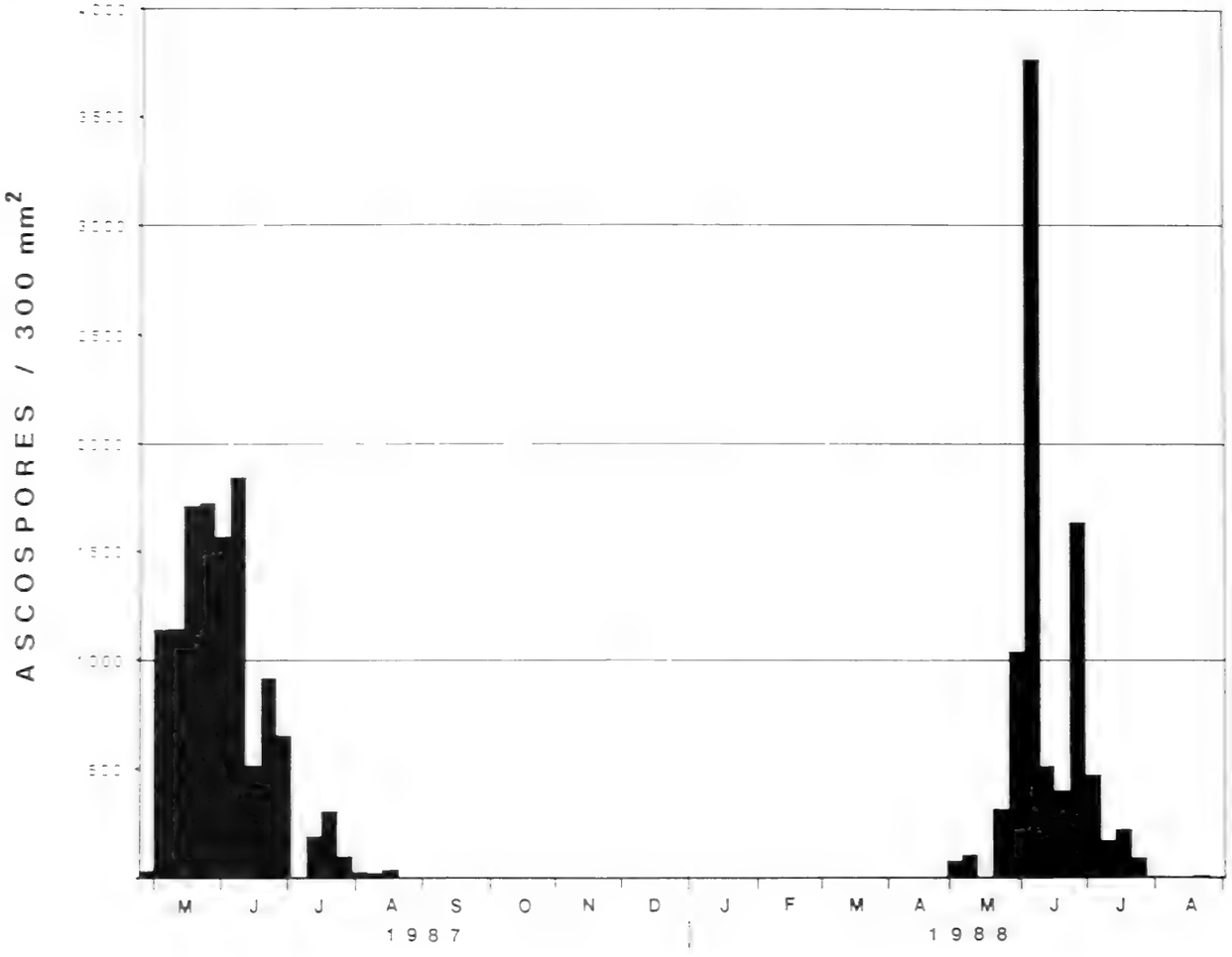
V = maturity of apothecia and ascospore discharge

VI = deterioration of apothecia and needles

Ap = apothecium
As = ascus
Asp = ascospores
Ka = conidiomata (acervuli)
Kn = conidia
Pa = paraphyses

seasons:
F = spring
S = summer
H = autumn
W = winter

Fig. 2. Number of ascospores of Lophodermium piceae captured on spore traps in 1987 and 1988.



Maturation of Apothecia and Control of Rhabdocline Needlecast on Douglas-Fir in Western Washington[2]

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Abstract.—Samples of one-year-old Douglas-fir (*Pseudotsuga menziesii*) needles were collected from Christmas trees in a plantation in western Washington from May through July in 1982 and 1983. Squash mounts were utilized to determine maturation of *Rhabdocline pseudotsugae* subsp *pseudotsugae* apothecia. Ascospores were found to be present in samples throughout the collection period. The highest percentage of asci with ascospores occurred in early June in 1982 and mid-May in 1983. Although ascospores were observed in squash mounts after mid-July in 1982, the numbers of apothecia discharging ascospores after moistening decreased rapidly after mid-July. To determine when needles become infected, trees were introduced or removed during May through June in 1982 and 1983 from a spray program using fungicides known to be effective in controlling Rhabdocline needlecast. Infection occurred during shoot elongation between 19 May and 16 June in 1982 and 19-25 May in 1983. The effectiveness of mancozeb, benomyl, and chlorothalonil fungicides in controlling Rhabdocline needlecast varied depending upon rates, application timing and disease pressure.

Introduction

Rhabdocline needlecast occurs on Dougles-fir (*Pseudotsuga mensiesii* (Mirb.) Franco) throughout its natural range and has been reported in areas where the host has been introduced (1,4,5). It has been known in the Pacific Northwest since 1917 where it can cause considerable damage on Douglas-fir being grown as Christmas trees (8,9,13,18,19).

Parker and Reid (15) revised the monotypic genus *Rhabdocline* Syd. to include two species, differentiated by the presence (*R. weirii* Parker and Reid) or absence (*R. pseudotsugae* Syd.) of an apical pore amyloid reaction, and five subspecies. Subspecies are separated by differences in the location of apothecia on needles (epiphyllous or hypophyllous), by constant association with a conidial anamorph (*Rhabdogloeum* spp.), and by ascospore and paraphysis morphology.

Control of this disease in Christmas tree plantations is based on planting selections with resistance to this disease, and the identification and removal of individual trees which are highly susceptible within plantations. If a susceptible selection is planted and grown during years in which Rhabdocline needlecast is epidemic, fungicides may be needed to control disease development.

Although recent work has been done on the chemical control of this disease in Michigan (12), information on the control of this disease on Douglas-fir Christmas trees in western Washington is lacking. This paper reports the results of experiments dealing with *Rhabdocline* apothecia maturation, identification of infection periods and fungicidal control of this disease in western Washington.

Materials and Methods

All work was done in a planting of approximately 1000 sheared Douglas-fir Christmas trees of unknown seed source near Olympia, Washington. Trees were 2-2.5 m tall, planted on 1.8 m centers and had a history of *Rhabdocline* damage.

Apothecia maturation and discharge of ascospores.—Procedures used to monitor the maturation of pseudothecia of *Venturia inaequalis* (Cke.) Wint. (6,17) were modified and used to assess maturation of *Rhabdocline* apothecia. During May through July in 1982 and 1983, ten trees were randomly selected and one twig was removed 1 m above ground on the north

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side of each tree. Twigs were labelled, bagged and stored at 5 C. Several 1-year-old needles bearing apothecia were randomly selected from each twig. Each needle was examined at 20X to determine if apothecia were hypophyllous or ephiphyllous. Five randomly selected apothecia on these needles were removed, and a squash mount in Melzer's reagent was made from each apothecium and examined for the presence (J+) or absence (J-) of an amyloid reaction of the ascus spore apparatus. This amyloid reaction is the primary means by which the two species of *Rhabdocline* are differentiated (*R. weirii* = J+, *R. pseudotsugae* = J-) (15). To determine apothecial maturity, 20 randomly selected asci per apothecium were examined and placed into one of three categories: 1) asci containing cytoplasm, 2) asci containing differentiated ascospores, and 3) empty asci.

During 1982, 10 randomly selected 1-year-old needles with apothecia from each twig were also mounted above 2 percent water agar in plates, moistened and incubated at 20 C (10). Ascospore discharge was noted by examining the surface of the medium below each set of needles for the presence or absence of ascospores 24 h later.

Ambient temperature and precipitation data during 1982 and 1983 were obtained from a weather station located approximately 4 kilometers from the test plot.

Identification of infection period.—Cover sprays of protectant fungicides known to control Rhabdocline needlecast were used during this study to protect trees during May and June in order to determine the infection period. During 1982 (benomyl, 1.1 kg ai/ha) and 1983 (chlorothalonil, Bravo 500 F, 4.7 kg ai/ha) sprays were applied to trees approximately every two weeks from mid-May through June. To determine infection periods, trees were added and removed from the spray program at each application. Applications were made with a Solo (Model 425) backpack sprayer equipped with a single 8003 LP teejet nozzle at 1.05 kg/m^2.

The experimental design was randomized complete blocks with 8 blocks. Treatments were applied to single trees within each block, and treated trees were separated from each other by one or more unsprayed trees. The length of a single current season terminal shoot was recorded for each of 10 randomly selected trees at each application date. The shoots were measured from the base of the bud on a lateral branch about 1.5 m. above ground in each quadrant of the tree.

The following spring, disease incidence was rated on a scale of 0-10 where 0 = no disease, 1 = 1-10%, and 10 = 91-100% symptomatic needles. Ratings were made for each quadrant of each tree on 4 May 1983 and 7 and 8 May 1984.

Effectiveness of various fungicides.—During 1982 and 1983, the relative effectiveness of benomyl, mancozeb (Fore 80W) and chlorothalonil in controlling Rhabdocline needlecast was determined. In each year, plots were set up as randomized complete blocks with 9 blocks. Treatments were applied to single trees in each block as described above. In 1982, benomyl at 0.6 and 1.1 kg ai/ha, mancozeb at 1.8 and 3.6 kg ai/ha and chlorothalonil at 2.3 and 4.7 kg ai/ha were applied on 19 May, 2 June and 16 June. In 1983, single applications of benomyl (1.1 kg ai/ha), mancozeb (3.6 kg ai/ha) and chlorothalonil (4.7 kg ai/ha) were applied to trees on 11 May. Applications of benomyl, mancozeb and chlorothalonil at half the above rates were also applied on 11 and 25 May. Disease evaluations were made on 7 and 8 May 1984 by rating disease incidence on a scale of 0-10 as described above.

Results

Apothecia maturation and discharge of ascospores.—During these studies, 569 apothecia from one-year-old needles were examined. All of the apothecia were J- and most were hypophyllous with filamentous paraphyses, indicating that *R. pseudotsugae* subsp. *pseudotsugae* was the predominant *Rhabdocline* taxon present. Although *R. weirii* subsp. *weirii* was not observed, *Rhabdogloem pseudotsugae* Syd., which is reported to occur in association with this taxon (15), was observed on a limited number of needles. Conidia from some *Rhabdogloem* acervuli lacked hyaline filiform appendages as described for *R. pseudotsugae* (15). *Rhabdogloem* acervuli were observed on the same needles with *R. pseudotsugae* subsp. *pseudotsugae* apothecia, but were not observed in the same lesions. *Rhabdocline pseudotsugae* subsp. *pseudotsugae* was also the only *Rhabdocline* taxon observed on a limited number of two-year -old needles examined during 1983.

Asci with differentiated ascospores were present during each sample period and the highest percentage of asci with ascospores occurred on 2 June in 1982 and 18 May in 1983 (fig. 1). Apothecia were initially orange-colored, but turned dark brown to black by late June in each year. By late June, less than 20% of the asci contained ascospores, and asci and ascospores were frequently distorted. *Cladosporium* spp. were associated with deteriorating apothecia during late June through the end of the sampling period. Except for samples collected right after a rain on 1 July, apothecia on moistened needles suspended above water agar released ascospores from all samples collected through 14 July, 1982. Only 10% of the samples collected on 29 July released ascospores, and none were released from samples collected on 12 August.

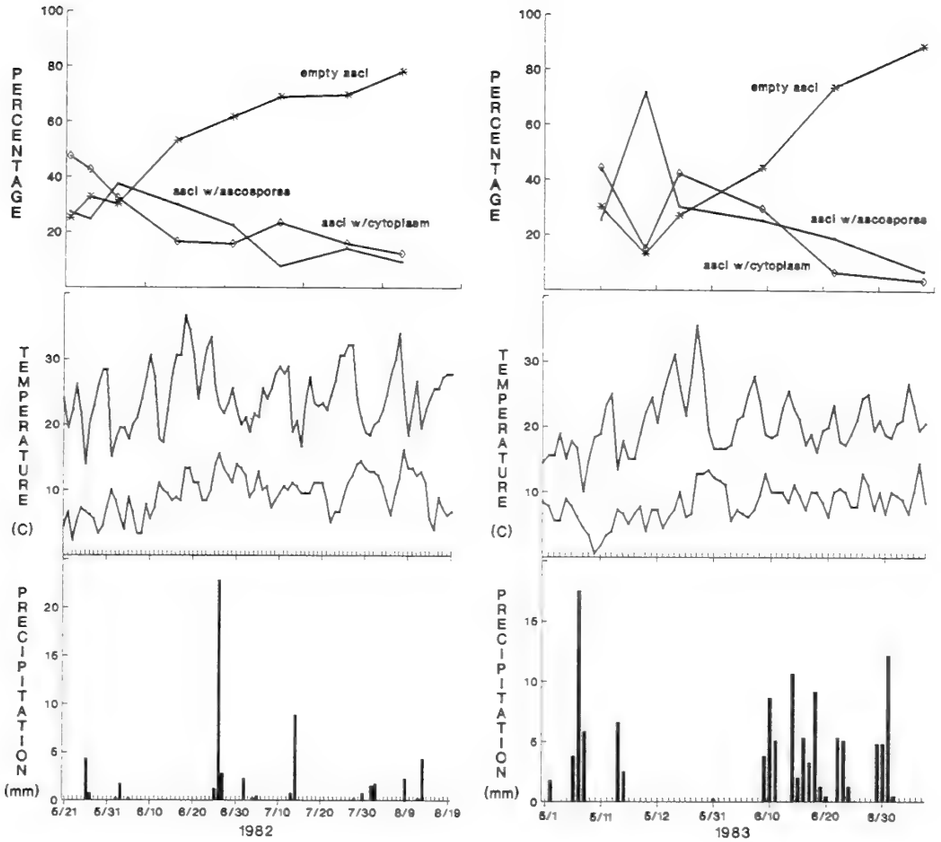

Figure 1.—Percentages of *Rhabdocline pseudotsugae* subsp *pseudotsugae* asci which contained ascospores, cytoplasm, or were empty and daily maximum-minimum temperatures and precipitation during 1982 and 1983.

Identification of infection periods.—By comparing the disease ratings for trees receiving various systematic fungicide applications during 1982 and 1983, we found that the majority of infections occurred during shoot elongation from late May through mid-June in 1982 and during late May in 1983. Trees which did not receive protective sprays between 19 May and 16 June in 1982 and 19-25 May in 1983 had disease ratings which were not significantly different from the nonsprayed checks (table 1). During these infection periods, shoots elongated from 3 to 11 cm in 1982 and 10.5 to 15.2 cm in 1983 (table 1).

Effectiveness of various fungicides.—In 1982, three applications of either benomyl, mancozeb or chlorothalonil provided effective disease control (table 2). There was no significant difference in the level of disease control obtained between any of the materials tested, and doubling the rates did not improve the level of disease control (data not shown). In 1983, single and double applications of these three fungicides were tested. Disease pressure was lower in 1983 than 1982 and a single application of mancozeb at 2.8 gm a. i./l or chlorothalonil at 5.0 gm a. i/l significantly reduced the level of disease compared to the nonsprayed check (table 3). The early single application of these fungicides was as effective as two applications of these materials at lower rates under this low disease pressure (table 3). During 1983, a single application of benomyl at 1.2 gm a. i./l was not effective and two applications at lower rates were only moderately effective (table 3).

Discussion

When the three parameters necessary for disease development (inoculum, susceptible host and favorable environment) are present for a sufficient period of time, disease will occur. Inoculum in the form of *Rhabdocline* ascospores is potentially present in Douglas-fir Christmas tree plantations in western Washington from the beginning of bud break through July (fig. 1), with the greatest potential closer to bud break. Similar observations are reported from Michigan (12).

Our observations would support the hypothesis that only young immature needles are susceptible to Rhabdocline infections. In both years of this study,

Table 1.—Initiation of cover sprays during 1982 and 1983 and control of Rhabdocline needle cast on Douglas-fir Christmas trees

1982[a]						1983[a]					
May		June			Disease	May			June		Disease
19	26	2	16	30	rating[b]	10	19	25	8	21	rating[b]
+	+	+	+	+	0.9 a	+	+	+	+	+	0.3 a
-	+	+	+	+	1.7 a	-	+	+	+	+	0.5 a
-	-	+	+	+	2.3 a	-	-	+	+	+	0.8 a
-	-	-	+	+	2.4 a	-	-	-	+	+	1.7 ab
-	-	-	-	+	2.7 ab	-	-	-	-	+	3.1 b
-	-	-	-	-	4.5 b	-	-	-	-	-	2.9 b
+	-	-	-	-	1.8 a	+	-	-	-	-	1.5 ab
+	+	-	-	-	1.6 a	+	+	-	-	-	0.1 a
+	+	+	-	-	2.1 a	+	+	+	-	-	0.0 a
+	+	+	+	-	0.9 a	+	+	+	+	-	0.4 a
Shoot development (percentage of final shoot length)[c]											
26.0	43.7	63.9	93.2	100.0		23.6	35.9	52.1	81.8	100.0	

[a] Cover sprays of benomyl (1982, 1.1 kg ai/ha) and chlorothalonil (1983, 4.7 kg ai/ha) were applied in the equivalent of 935.4 l of water per hectare. + = tree sprayed and - = tree not sprayed.

[b] Average disease rating for 8 trees per treetment. Disease ratings based on a scale of 0-10, where 0 = no disease and 10 = 100% of the needles diseased. Ratings for 1982 cover sprays made on 4 May 1983 and those for 1983 cover sprays made on 7-8 May 1984. Numbers in vertical columns followed by the same letter are not significantly different, P = 0.05, Duncan's multiple range test.

[c] The length of a single current season terminal shoot on a lateral branch about 1.5 m above ground in each quadrant for 10 randomly selected trees was measured and used to calculate the rate of shoot development.

Table 2.—Effectiveness of fungicides in controlling Rhabdocline needlecast on Douglas-fir

Treatment[a]	g. ai/l	Disease rating[b]
Check	-	3.0 a
Benlate 50W	0.6	0.8 b
Fore 80W	1.8	0.7 b
Bravo 500	2.3	0.2 b

[a] Fungicides were applied to trees on 19 May, 2 June and 16 June 1982 in the equivalent of 935.4 l of water per hectare.

[b] Average disease rating for 9 trees per treatment on 4 May 1983. Disease rating based on a scale of 0-10 where 0 = no disease and 10 = 100% of needles with symptoms. Numbers followed by the same letter are not significantly different, P = 0.05, Duncan's multiple range test.

Table 3.—Effect of fungicides and number of applications on the control of Rhabdocline needlecast on Douglas-fir

Treatment[a]	Rate g ai/l	No. of applications	Disease rating[b]
Check	-	-	1.17 ab
Benlate 50W	1.2	1	1.97 a
Benlate 50W	0.6	2	0.86 bc
Manzate 80W	2.8	1	0.11 c
Manzate 80W	1.9	2	0.11 c
Bravo 500	5.0	1	0.22 c
Bravo 500	2.5	2	0.22 c

[a] Fungicides were applied to trees on 11 May (single) or 11 May and 25 May (double) 1983 in the equivalent of 935.4 l of water per hectare.

[b] Average disease rating for 9 trees per treatment on 7 and 8 May 1984. Disease rating based on a scale of 0-10 where 0 = no disease and 10 = 100% of needles with symptoms. Numbers followed by the same letter are not significantly different, P = 0.05, Duncan's multiple range test.

rainy periods occurred either in late June or July when ascospores were present (fig. 1). However, no apparent infections took place during these times (table 2). The weather conditions during the last 10 days in June in 1983 were particularly favorable for disease, and yet there was no beneficial response to fungicide applications at that time (table 1).

Prolonged periods of high relative humidity and mild temperatures favor infection and disease development (14). Weather data suggest that three and two infection periods occurred during the period of needle elongation and relatively high inoculum levels during 1982 and 1983, respectively. Thus, as might be anticipated, disease incidence was greater in 1982 than 1983.

Results from the 1983 fungicide trial suggest only one application of an effective fungicide will adequately control *Rhabdocline*; however, during the time of the second application on May 25, the weather conditions were not favorable for disease and no response would be expected (fig. 1). The application made several weeks later on June 8 in 1982 was effective, indicating that, in some years, a second application may be required.

Christmas tree growers commonly apply one of the three fungicides we tested above to control Swiss needlecast disease (2,7,11,16). One application during the period from bud break to when new shoots are 2.5 to 5 cm long has given excellent control in Washington and Oregon (2,3,7). Results from this study indicate that this application will also control early infections of Rhabdocline. If wet weather persists and inoculum levels are high from previous years' infections, a second application 3 to 4 weeks later should improve the level of control.

Acknowledgements

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Literature Cited

1. Brandt, R. W. 1960. The Rhabdocline needle cast of Douglas-fir. SUNY Coll. Forestry Tech. Bull. 84, 66 p.
2. Chastagner, G. A., Byther, R. S. 1983. Control of Swiss needle cast on Douglas-fir Christmas trees with aerial appliations of chlorothalonil. Plant Dis. 67:790-792.
3. Chastagner, G. A., Byther, R. S.. 1983. Infection period of *Phaeocryptopus gaeumannii* on Douglas-fir needles in western Washington. Plant Dis. 67:811-813.
4. Chen, R. C. 1972. Adelopus needle cast disease of Douglas-fir in central New York. Exp. For. Nat. Univ. Taiwan Tech. Bull 103.
5. Ellis, D. E., Gill, L. S. 1945. A new *Rhabdogloeum* associated with *Rhabdocline pseudotsugae* in the southwest. Mycologia 37:326-332.
6. Gadoury, D. M., MacHardy, W. E. 1982. Preparation and interpretation of squash mounts of speudothecia of *Venturia inaequalis*. Phytopathology 72:92-95.
7. Hadfield, J., Douglass, B. S. 1982. Protection of Douglas-fir Christmas trees from Swiss needle cast in Oregon. Am. Christmas Tree J. 26(2):31-33.
8. Maloy, O. C., Partidge, A. 1970. Christmas tree diseases. Wash. State Univ. Coop. Ext. Bull. 606. 12 p.
9. McDowell, J., Merrill, W. 1985. *Rhabdocline* taxa in Pennsylvania. Plant Dis. 69:714-715.
10. Michaels, E., Chastagner, G. A. 1984. Seasonal availability of *Phaeocryptopus gaeumannii* ascospores and conditions that influence their release. Plant Dis. 68:942-944.
11. Morton, H. L. 1975. Biology and control of Swiss needle cast. (Abstr.) Proc. Am. Phytopath. Soc. 2:30.
12. Morton, H. L., Miller, R. E. 1982. Chemical control of Rhabdocline needle cast on Douglas-fir. Plant Dis. 66:999-1000.
13. O'Brien, J. G., Morton, H. L. 1983. Occurrence of *Rhabdocline* taxa in Douglas-fir tree plantations in Michigan. Plant Dis. 67:661-663.
14. Parker, A. K. 1970. Effect of relative humidity and temperature on needle cast disease of Douglas-fir. Phytopathlogy 60:1270-1273.
15. Parker, A. K., Reed, J. 1969. The genus *Rhabdocline* Syd. Can. J. Bot. 47:1533-1545.
16. Skilling, D. D. 1981. Control of Swiss needle cast in Douglas-fir. Am. Christmas Tree J. 25(3):34-37.
17. Szkolnik, M. 1969. Maturation and discharge of ascospores of *Venturia inaequalis*. Plant Dis. Rep. 53:534-537.

18. Weir, L. C. 1917. A needle blight of Douglas-fir. J. Agric. Res. 10:99-103.

19. Wiestaner, D. A. 1955. Some studies on *Rhabdocline pseudotsugae* Syd. in western Montana. Unpublished M.S. Thesis. Montana State Univ., Bozeman. 83 p.

Rhabdocline Needlecast Resistance in Douglas-Fir Seed Sources From the Southwestern United States [1,2,3]

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Abstract.—Rhabdocline needlecast resistance was evaluated among the progeny of 71 individual Douglas-fir mother trees from 16 localities in Arizona and New Mexico and one in Colorado. Progeny of mother trees from New Mexico were significantly more resistant than those from Arizona. Progeny of mother trees from two localities in the Carson and Santa Fe National Forests were the most resistant. Resistance was strongly correlated with longitude, weakly correlated with elevation, and not correlated with latitude of the source.

Rhabdocline needlecast, caused by the fungi *Rhabdocline pseudotsugae* Syd. and *R. weirii* Parker & Reid, is the major disease affecting production of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] Christmas trees in the northeastern United States (5). European researchers in the 1930's (2, 3, 10) and others since that time (1, 7, 8, 9) have shown considerable variation in Rhabdocline needlecast resistance among different provenances and strains of Douglas-fir. Most of these investigators used seed collections pooled from several trees, or often taken from squirrel caches. In general, southwestern United States and Rocky Mountain provenances are the most susceptible while the coastal variety from the Pacific Northwest is the most resistant. Yet, southwestern provenances are favored for Christmas tree production in the northeastern United States due to their rapid growth rate and tolerance to frost and winter injury (1). An estimated 85 percent of the more than 4100 hectares of Douglas-fir Christmas trees in Pennsylvania is from seed sources in the Lincoln National Forest (NF) in New Mexico.

In 1975, one of several plantations in a Douglas-fir provenance study was established near University Park, Pennsylvania to evaluate, for Christmas tree production, the growth rate, frost tolerance, form, and color of various Douglas-fir seed sources located in the southwestern United States. The plantation consisted of four blocks, each block containing four progeny from each of 107 mother trees. These mother trees were from widely scattered localities from the Kaibab NF near the Utah border to the Coronado NF close to the Mexican border in Arizona, from the Carson NF near Taos south to the Gila NF near Silver City in New Mexico, and from several other localities.

In May 1983, needlecast caused by *R. pseudotsugae* subsp. *pseudotsugae* Parker & Reid, the most common cause of Rhabdocline needlecast in Pennsylvania (4), was found generally distributed throughout the plantation. By 1986, disease incidence had increased so that many trees lost more than 50 percent of their 1985 needle complement on branches within 1.5 m of the ground. However, some trees remained disease-free even though their branches intertwined with those of infected trees. This provided the opportunity to evaluate these trees for resistance to Rhabdocline needlecast under natural infection in a climatic regime typical of much of the production area in the northeastern United States (5). A preliminary note as been published (6).

Materials and Methods

On 1 May 1986, all progeny were examined and rated for disease severity using a scale where 1 = uninfected, 2 = <50% of the needles infected on branches within 1.5 m of the ground, and 3 = >50% of the needles infected on branches within 1.5 m of the ground. Numerous trees within the plantation were missing due to various causes. From 5 to 37 trees per locality remained; 326 trees, the progeny of 71 mother trees from 17 localities, were included in the analysis. Separations of mean disease ratings were made by applying Student's "t" test between pairs of means at $P = 0.05$. The elevation, latitude, longitude, number of mother trees, number of progeny and average

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Table 1.—Average Rhabdocline needlecast disease rating for 326 progeny from 71 Douglas-fir mother trees from 17 localities in the southwestern United States, with elevation, latitude and longitude of each locality

National Forest and Locality[x]	State[y]	Elevation of Locality meters	Latitude of Locality °N	Longitude of Locality °W	Number of Parent Trees	Number of Progeny Tested	Average Disease Rating[z]
Santa Fe HGCG	NM	2393	35.77	105.67	6	30	1.1 a
Carson DC	NM	2895	36.08	105.23	3	9	1.1 ab
Santa Fe BM	NM	2560	35.97	105.80	2	5	1.5 abc
Cibola SMt	NM	3308	35.20	106.42	5	28	1.6 bc
Carson GCG	NM	2312	36.70	105.52	5	27	1.9 cd
Coronado Chir	AZ	1895	31.90	109.23	5	37	2.0 cd
AFA	CO	2286	39.00	104.00	4	23	2.0 cd
Gila MR	NM	2012	33.40	108.77	5	26	2.0 cd
Cibola MMt	NM	2500	34.05	107.20	5	21	2.0 cd
Gila CC	NM	2103	32.92	108.20	5	21	2.2 de
Tonto PMt	AZ	2347	32.38	110.68	4	17	2.2 def
Tonto EPCG	AZ	1585	34.30	111.05	3	10	2.3 def
Lincoln SB	NM	2743	33.42	106.00	4	8	2.4 def
Coronado MtL	AZ	2316	32.28	109.23	7	29	2.5 ef
Coconino SB	AZ	2743	35.32	111.70	3	11	2.5 ef
Coconino OCC	AZ	1524	34.97	111.75	3	18	2.8 f
Kaibab GH	AZ	2500	35.35	111.95	2	6	3.0 f

[x] Letters after the name of the National Forest are coded for specific locations within that forest.
[y] AZ = Arizona, CO = Colorado, NM = New Mexico.
[z] Average disease ratings followed by the same letter not significantly different at $P = 0.05$ (Student"s "t"). 1 = no needlecast; 2 = <50% of needles <1.5 m from ground infected; 3 = >50% of needles <1.5 m from ground infected.

disease rating for the progeny from each locality are listed in table 1. The letters following the name of the National Forest in which the mother trees were located are a coded designation for a specific site within that forest.

Results and Discussion

Trees from two localities, Santa Fe HGCG and Carson DC, had a high degree of resistance to Rhabdocline needlecast (table 1). Progeny from other localities were moderately to very susceptible. When data for the Santa Fe HGCG and Carson DC localities were pooled, the average disease rating was significantly lower than those of all other sources ($P < 0.0005$). Data from the Santa Fe BM locality are "soft" due to the small number of parent trees and progeny involved.

When all data were pooled by state, progeny of New Mexico trees were significantly more resistant than those of Arizona trees ($P < 0.0005$). The latter is important to growers who purchase seedlings labeled "from Arizona seed sources" or "from New Mexico seed sources", as well as to nurserymen who purchase bulk seed lots similarly labelled. Stephan (8), working with a different collection of seed in northwestern Germany, also found that trees originating from New Mexico seed sources were more resistant than those originating from Arizona, Utah, or Colorado sources.

Average disease rating was not correlated with latitude of the source ($R^2 = 0.09$, $P = 0.17$). Average disease rating was weakly correlated with elevation of the source ($R^2 = 0.25$, $P = 0.08$), with lower disease ratings with increasing elevation. Average disease rating (Y) was strongly correlated with longitude of the source (X), with higher disease ratings with increasing longitude: $Y = -13.7 + 0.1X$ ($R^2 = 0.49$).

In 1932, Liese (3) noted that although Douglas-firs from southwestern United States provenances generally were very susceptible to Rhabdocline needlecast, individual highly resistant trees occurred within progeny from these sources. Jaynes, et al (1) also found that although trees from southwestern sources generally were quite susceptible, trees from a Santa Fe NF source were as resistant as those from some Pacific Northwest sources. The data of Jaynes, et al (1)

also showed a trend for increasing susceptibility with increasing longitude of the southwestern sources.

Douglas-fir has a discontinuous distribution in the southwestern United States, being restricted primarily to sheltered canyons or to higher elevations where there is sufficient precipitation to support its growth. Large zones of other vegetation types intervene between these areas. Apparently within some of these isolated "island" populations of Douglas-fir, inbreeding within restricted groups of individuals has given rise to a series of localized populations somewhat distinct from each other. For example, in the Santa Fe HGCG locality, which consisted of six mother trees scattered over a distance of 1.6 km, all parents yielded progeny with similar levels of disease resistance. Likewise, in the Coronado MtL locality, six mother trees located several kilometers apart yielded progeny with similar levels of disease resistance. Thus, Rhabdocline needlecast resistance appears to be characteristic of some small subpopulations of southwestern Douglas-fir. These can provide excellent seed sources for Christmas tree production in the northeastern United States if disease resistance can be coupled with frost tolerance and with good growth rate, form, and color. In selecting seed sources for Christmas tree improvement, it is important to concentrate on specific localities and individual mother trees rather than on pooled seed collections made over extensive areas or taken from squirrel caches.

Literature Cited

1. Jaynes, R.A., Stephens, G.R., Ahrens, J.F. 1986. Douglas fir seed sources tested for Christmas trees in Connecticut. Am. Christmas Tree J. 30(1):12-14.
2. Liese, J. 1931. Zur Rhabdoclinekrankheit der Douglasie. Forstarch. 7:341-346.
3. Liese, J. 1932. Zur Biolgie der Douglasiennadelschütte. Ztschr. f. Forst- u. Jagdwesen 64:680-693.
4. McDowell, J., Merrill, W. 1985. *Rhabdocline* taxa in Pennsylvania. Plant Dis. 69:714-715.
5. Merrill, W., Cameron, E.A. 1986. Christmas tree pests and pest management in the Northeast. Pa. Agric. Exp. Sta. Prog. Rep. 388, 35 p.
6. Merrill, W., Wenner, N.G. 1987. Resistance of Douglas-fir to Rhabdocline needlecast and Cooley's adelgid. (Abstr.) Phytopathology 77:120.
7. Schober, R., Meyer, H. 1955. Douglasien-Provenienzversuche. 2. Allg. Forst- u. Jagdztg. 126:221-243.
8. Stephan, B.R. 1973. Über Anfälligkeit und Resistenz von Douglasien= Herkünften gegenüber *Rhabdocline pseudotsugae* . Silvae Genetica 22:149-153.
9. Stephan, B.R. 1980. Prüfung von Douglasien-Herkunften auf Resistenz gegen *Rhabdocline pseudotsugae* in Infektionsversuchen. Eur. J. For. Path. 10:152-161.
10. van Vloten, H. 1932. *Rhabdocline pseudotsugae* Sydow, oorzaak eener ziekte van Douglasspar. Thesis, Wageningen Agricultural College. 168 p. (Rev. Appl. Mycol. 12:63-64, 1933)

Control of Melampsora Needle Rust on Douglas-Fir Christmas Trees[1,2]

Gary A. Chastagner[3], John M. Staley[3], and William M. Proebsting[4]

Abstract.—Needle rust, caused by *Melampsora occidentalis*, can severely damage Douglas-fir Christmas trees in areas where poplars, the alternate host, cannot be removed. Alternatives for controlling this disease in these areas would be to use Douglas-fir resistant to *M. occidentalis* or to protect susceptible trees with fungicides. The results of a study to determine the potential for host resistance to control this disease clearly showed that there were significant differences in the susceptibility of the 28 2-yr-old clones of Douglas-fir tested. The average number of needles with rust and needle scars for individual clones ranged from 0.8 to 22.2 and 0.0 to 20.6 per 5 cm of shoot, respectively. Nineteen experimental and/or commercial fungicides were also tested for their ability to control rust development. All of these materials significantly reduced the level of rust compared to the nonsprayed check when a single application was applied during early shoot development.

Introduction

Melampsora occidentalis Jackson is a heteroecious, macrocyclic rust which causes needle rust on Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) in the Pacific Northwest (16,17,18). Although Douglas-fir is the most common coniferous host of this pathogen in the Pacific Northwest, Ziller (17) showed that a number of larch and pine species are also susceptible to *M. occidentalis*. Poplars serve as the alternate host of this rust and black cottonwood (*Populus trichocarpa* Torr. & Gray) is the most common poplar host in western North America (18).

This rust overwinters as telia on dead poplar leaves. During the spring, basidiospores are wind-disseminated to developing needles. Pycnia and then aecia develop within about 2 weeks on slightly chlorotic areas on infected needles. These discolored areas become necrotic, and the needles shrivel and are shed after aecial production. The aeciospores are wind dispersed to infect poplar leaves where yellow uredinia appear within 2 weeks. The number of uredinia and infected leaves increase during the summer as urediniospores reinfect poplar. During late summer, dark brown crust-like telia form in place of the uredinia and overwinter on fallen leaves to complete the life cycle of the rust. The severity of rust which develops on the coniferous and poplar hosts depends on the proximity of these hosts to each other, the level of inoculum, the susceptibility of each host to the rust, and environmental conditions.

During June 1984, 1985, and 1988, Melampsora needle rust severely damaged Douglas-fir Christmas trees in plantations in the Puget Sound area of western Washington. In addition to the rust symptoms on needles described above, stem lesions were associated with rusted needles (3). In one plantation of about 1,000 1.5-2.0 m tall trees, approximately 400 trees with needle rust and stem lesions were found within 40 m of a black cottonwood tree. In this plantation, lesion development distorted or killed entire shoots on many of the 400 severely rusted trees.

Although considerable work has been done on the control of poplar leaf rusts caused by various *Melampsora* spp., little information is available regarding the control of *M. occidentalis* on Douglas-fir. Control is primarily based on the removal of the poplar hosts in the immediate vicinity of Christmas tree plantations. However, in some instances this is not possible.

Host resistance is the principal means of controlling Melampsora leaf rust on poplars (6,7,8,9,13,14,15), and observations within Douglas-fir Christmas tree plantations indicate there is considerable variation in

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the susceptibility of Douglas-fir seedlings to Melampsora needle rust. Commercial asexual production of Douglas-fir using cuttings makes it possible to propagate clones of Douglas-fir with desirable Christmas tree characteristics combined with resistance to pest and disease problems (11,12). A number of Douglas-fir clones with desirable horticultural characteristics for use as Christmas trees have been identified and are being commercially produced in the Pacific Northwest. By screening these clones for their susceptibility to Melampsora needle rust, we may identify sources of resistance to this needle rust for use in areas where cottonwoods cannot be removed.

Another method of controlling Melampsora needle rust is to protect susceptible trees with fungicides during periods favorable for infection. A number of fungicides have been shown to control Melampsora leaf rust on poplar (10,15), but little information is available regarding the effectiveness of fungicides in controlling Melampsora needle rust on Douglas-fir.

This paper reports the results of studies to determine the susceptibility of select Douglas-fir clones to Melampsora needle rust and the effectiveness of fungicides in protecting Douglas-fir from this disease.

Materials and Methods

Susceptibility of clones.—To determine the susceptibility of Douglas-fir clones to Melampsora needle rust, 28 2-year-old clones grown in 6.3 x 25.0 cm deep pots were exposed to overwintered cottonwood leaves with *M. occidentalis* teliospores. A single pot of each clone was randomly positioned in each of five groups (clones Douglass 1 and 2 were placed only in two and four groups, respectively) on May 5, 1988 at Washington State University's Farm 1 near Puyallup, Washington. To insure abundant levels of inoculum, overwintered cottonwood leaves with telia of *M. occidentalis* were collected from several areas within a 6.4 km radius of Puyallup and placed between two layers of 1.2 cm mesh netting approximately 25-30 cm above the five groups of potted clones. Daily overhead sprinkler irrigation was used to irrigate the clones and supplement natural precipitation to provide periods of free moisture necessary for basidiospore production and infection of developing 1988 needles.

To determine the susceptibility of each clone to *M. occidentalis*, a single 1988 shoot was collected from each exposed tree, placed in a labeled plastic bag and stored at 5C on 14-15 July, 1988. The number of needles with rust, needle scars and stem lesions were then counted along a 5.0 cm length of each shoot. Data were analyzed using ANOVA and Duncan's multiple range test to separate means.

Fungicidal control.—The effectiveness of several commercial and experimental fungicides in controlling needle rust on 0.4 to 0.6 m tall field-grown Douglas-fir was tested during 1985 and 1986. Anilazine (Dyrene 4F), benodanil (Benefit), bitertanol (Baycor 50W), chlorothalonil (Bravo 500), cyclohexamide (Actidione 5W), fenarimol (Rubigan 1F), ferbam (Carbamate WDG76/Carbamate 76W), mancozeb (Dithane M45 80W), propiconazol (Banner 1.1EC), triadimefon (Bayleton 25DF), triforine (Funginex 1.6EC/Triforine 18.2EC); zineb (Dithane Z-78 75W); zyram (Zyram 76W/Z-C Spray 76W), diniconazole (Spotless 25W), myclobutnanil (Systhane 40W), SN-84364 50W (Nor Am), and SN-596 50W (Nor Am) fungicides were mixed into water containing 2.5 ml of Ortho X-77 per 4 l of water. Treatments were applied to individual trees using a Solo hand pump backpack sprayer (Model 425) equipped with a single teejet 8003LP nozzle at a pressure of 1.05 kg/cm^2. Trees were sprayed to runoff on 17 May 1985 and 16 May 1986 when new growth averaged 3 cm and 4.1 cm, respectively. Nonsprayed trees served as checks. Plot designs were randomized complete blocks with nine (1985) or three (1986) blocks.

One to 3 days after fungicide application, overwintered cottonwood leaves infected with *M. occidentalis* were suspended approximately 2 to 5 cm above the trees to provide basidiospore inoculum. Disease development was determined by collecting a current season shoot from each tree on a July 1985 and 23-24 June 1986 and counting the number of infected needles. Data were analyzed using analysis of variance and Duncan's multiple range test to separate means.

Results and Discussion

Susceptibility of clones.—As reported by Ziller (16,17,18), symptom development on clones exposed to *M. occidentalis* inoculum consisted of needles with pycnia and aecia later becoming necrotic and dropping off. The average number of needles with *M. occidentalis* and the number of needle scars for individual clones ranged fro 0.8 to 22.2 and 0.0 to 20.6, respectively. The data for number of needles with rust and needle scars on individual samples were combined to assess the relative susceptibility of the clones to *M. occidentalis*. Since these data appeared to have a poisson distribution, the data were transformed as square root of (X + 1/2) prior to analysis. There were significant differences in the susceptibility of the 28 clones exposed to *M. occidentalis* (table 1). Clones Douglass 7, 28 and 19 had significantly higher levels of rust than Douglass 1, 6, 8, 10, 13, 17, 23, 24, 25, 27, Newton, Mitchell 1 and Kintigh 21-2. Whether these least susceptible clones will maintain their relative degree of resistance under more natural or

Table 1.—Susceptibility of 28 Douglas-fir clones to *Melampsora occidentalis*

Clone[1]		Rust level[2]	
Douglass	7	28.0	a
Douglass	28	27.1	a
Douglass	9	25.8	ab
Douglass	12	20.6	abc
Douglass	2	20.3	abc
Douglass	20	17.4	abcd
Douglass	18	15.2	abcde
Douglass	21	15.0	abcde
Douglass	19	14.6	abcde
Douglass	22	13.3	abcdef
Douglass	5	12.9	abcdef
Lee	3	12.1	abcdef
Hofert	3	10.5	abcdef
Douglass	14	10.3	abcdef
Douglass	15	8.9	bcdef
Douglass	8	8.5	cdef
Douglass	1	7.8	cdef
Mike Newton		7.4	cdef
Douglass	25	7.1	cdef
Douglass	27	6.9	cdef
Douglass	10	6.2	cdef
Douglass	6	5.3	def
Douglass	13	5.1	def
Douglass	24	5.0	def
Douglass	17	4.9	def
Douglass	23	4.9	def
A. Mitchell	1	2.8	ef
Kintigh	21-2	2.0	f

[1] A single pot of each 2-year-old clone was randomly placed in each of five groups (clones Douglass 1 and 2 were only placed in 2 and 4 groups, respectively) on May 5, 1988. Overwintered cottonwood leaves with telia of *M. occidentalis* were suspended above the groups of pots to provide uniform inoculum.

[2] Rust levels based on the number of needles with rust and needle scars on a 5.0 cm long portion of a single 1988 shoot per tree. Because data had a poisson distribution, the data were transformed as square root of (X+1/2) prior to analysis. Numbers followed by the same letter are not significantly different, P=0.05, Duncan's mulitple range test.

altered test conditions where a potentially more diverse genetic population of the pathogen exists is unknown.

Although individual Douglas-fir trees free of rust infection were observed among severely rusted trees in some plantations, none of the clones tested was completely resistant to *M. occidentalis*. Testing additional clones and/or taking cuttings from apparently rust resistant trees in plantations would probably identify material with higher levels of rust resistance than found in the 28 clones tested.

Stem lesions, which are associated with high levels of rust on Douglas-fir (3), were also observed on many of the clones. The average number of stem lesions ranged from 0.0 to 9.2 for individual clones. Although low, there was a significant positive correlation ($r = 0.39$, $P < 0.05$) between the numbers of stem lesions observed and the overall rust susceptibility of the clones.

Fungicides.—All of the fungicides tested significantly reduced the number of infected needles compared to the check (table 2). Of the fungicides tested, only Bravo 500 and Dithane M-45 are registered for use on Douglas-fir Christmas trees. Actidione applied at 0.03 mg ai/ml during 1985 was phytotoxic, killing 8 of 9 trees (data not shown).

The results of both of these studies indicated that clones being developed for use in the Christmas tree industry have varying levels of genetic resistance to *M. occidentalis*. Clones with higher level of resistance to *M. occidentalis* can probably be found by screening additional clones not included in our tests. Primary selection from rust-free trees in severely infected plantations would be a likely source of such material. Additional research is needed to determine the effectiveness of this resistance in minimizing damage to Douglas-fir grown as Christmas trees in areas adjacent to the alternate host in the Pacific Northwest. In the absence of complete genetic resistance, fungicides could be used. A number of fungicides were effective in controlling rust infection and subsequent stem lesion development. The partial resistance of some clones demonstrated in this study could be used to supplement the effectiveness of fungicides in controlling this disease. Recent work in western Washington has shown that infection of Douglas-fir needles by *M. occidentalis* occurs during a 2-3 week period from mid-May to early June during early shoot elongation (4). Thus for these fungicides to protect the new growth, they would need to be applied during the early stages of shoot development. In addition to controlling Melampsora needle rust, applications of fungicides such as chlorothalonil and mancozeb would also provide control of Swiss needlecast (1,2) and Rhabdocline needlecast (5). Copper-containing fungicides have also been shown to be effective in controlling rust on both Douglas-fir and cottonwood (10,15), but were not included in our test.

Table 2.—Effectiveness of a single fungicide application in controlling *Melampsora occidentalis* on Douglas-fir during 1985 and 1986

	1985		1986	
Treatment[1]	Rate mg ai/ml	Ave. no. of needles with rust/ cm of shoot[2]	Rate mg ai/ml	Ave. no. of needles with rust/ cm of shoot[2]
Check	-	3.6a	-	2.7a
Benefit 50W	1.2	2.1 b	1.80	0.0 b
Bravo 500	2.5	1.4 bc	1.32	0.7 b
Rubigan 1F	0.15	1.4 bc	0.15	0.1 b
Plantvax 75W	0.9	1.3 bc	-	-
Dithan Z-78 75W	1.8	1.3 bc	-	-
Dyrene 4F	2.4	1.2	-	-
Dithane M-45 80W	1.9	1.0 bc	1.91	0.0 b
Baycor 50W	0.3	0.3 c	-	-
Banner 1.1EC	0.16	0.3 c	-	-
Carbamate 76W	1.8	0.1 c	-	-
Carbamate WDG 76%	-	-	1.82	0.0 b
Z-C Spray 76W	1.8	0.1 c	-	-
Ziram 76W	-	-	1.82	0.0 b
Triforine 18.2EC	0.24	0.0 c	-	-
Funginex 1.6EC	-	-	0.24	0.0 b
Bayleton 25DF	0.3	0.0 c	0.06	0.0 b
Systhane 40W	-	-	0.12	0.2 b
Spotless 25W	-	-	0.03	0.0 b
SN84364 50W	-	-	0.60	0.0 b
SN596 25DF	-	-	0.15	0.0 b

[1] Fungicides were mixed into water containing 2.5 ml of X-77 per 4 l of water and individual 0.4-0.6 m tall field grown Douglas-fir were sprayed to runoff on 17 May 1985 and 16 May 1986 when new growth averaged 3 cm and 4.1 cm, respectively. Nonsprayed trees serves as checks and the plot designs were randomized complete blocks with 9 (1985) and 3 (1986) blocks.

[2] Disease data were collected on 2 July 1985 and 23-24 June 1986. Numbers followed by the same letter are not significanlty different, P=0.05, Duncan's multiple range test.

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Literature Cited

1. Chastagner, G. A., Byther, R. S. 1983. Control of Swiss needle cast on Douglas-fir Christmas trees with aerial applications of chlorothalonil. Plant Dis. 67:790-792.

2. Chastagner, G. A., Byther, R. S. 1983. Infection period of *Phaeocryptopus gaeumannii* on Douglas-fir needles in western Washington. Plant Dis. 67:811-813.

3. Chastagner, G. A., Staley, J. M., Byther, R. S. 1986. Melampsora stem cankers and fungicide control on Douglas-fir. (Abstr.) Phytopathology 76:1124.

4. Chastagner, G. A., Staley, J. M. 1988. Factors affecting primary inoculum production and infection of Douglas-fir by *Melampsora occidentalis*. Phytopathology 78:1613.

5. Chastagner, G. A, Byther, R. S., Riley, K. L. 1990. Maturation of apothecia and control of Rhabdocline needle cast on Douglas-fir in western Washington. p. 87-92. In: W. Merrill and M. Ostry (eds.). Recent Research on Foliage Diseases, USDA For. Serv. Gen. Tech. Rep. WO-56, 145 p.

6. Chiba, O. 1964. Nature of resistance of poplar clones to leaf rust, *Melampsora Larici-populina*. p. 207-220. In: H. D. Gerhold, et. al. (eds). Breeding pest resistant trees. Pergammon Press, Toronto. 505 p.

7. Cooper, D. T., Filer, T. H. 1977. Geographic variation in Melampsora rust resistance in eastern cottonwood in the lower Mississippi Valley. p. 146-151. In: Proc. 10th Central States Forest Tree Improvement Conf.

8. Eldridge, K., Matheson, A., Stahl, W. 1973. Genetic variation in resistance to poplar leaf rust. Australian Forest Res. 6:53-59.

9. F.A.O. 1986. Breeding Poplars for Disease Resistance. FAO Paper 56. F.A.O. Rome, Italy. 66 p.

10. Fuller, R. A., Menzies, S. A. 1974. Examination of fungicides for control of poplar leaf rust in shelter belts. N.Z. J. Exper. Agric. 2:429-431.

11. Proebsting, W. M. 1984. Rooting of Douglas-fir stem cuttings: relative activity of IBA and NAA. HortScience. 19:854-856.

12. Proebsting, W. M. 1983. Selection of Douglas-fir clones in the Pacific Northwest. Am. Christmas Tree J. 27:25-27.

13. Shain, L. 1976. Etiology, epidemiology and control of Melampsora rust of cottonwood. p. 189-198. In: B. Thielges and S. Land (eds). Symposium on eastern cottonwood and related species. Louisiana State Univ.

14. Spiers, A. G. 1974. Control of poplar leaf rust *Melampsora larici-populina* in New Zealand. N.Z. J. Exper. Agric. 2:433-436.

15. Widin, K. K., , A. L. 1981. Effect of Melampsora leaf rust infection on yield of hybrid poplars in the North Central U.S. Eur. J. For. Path. 11:438-448.

16. Ziller, W. G. 1955. Studies of western tree rusts. II. *Melampsora occidentalis* and *M. albertensis*, two needle rusts of Douglas-fir. Can. J. Bot. 33:177-188.

17. Ziller, W. G. 1965. Studies of western tree rusts. XI. The aecial host ranges of *Melampsora albertensis, M. medusae*, and *M. occidentalis*. Can. J. Bot. 43:217-230.

18. Ziller, W. G. 1974. The tree rusts of western Canada. Can. For. Serv. Publ. No. 1329, Victoria, BC. 272 p.

Comparative Histopathology of Pine Tissues Infected by Needlecast and Needle Blight Fungi [1,2]

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Abstract.—The five needle-inhabiting fungi studied produced similar host tissue abnormalities, although they eventually colonized different host tissues. Some of these pathogens were able to cause extreme host abnormalities with limited detectable colonization of the host. The spectacular amount of hyphal colonization by *Ploioderma hedgecockii* was very unusual in comparison with the other pathogens in their respective hosts, and especially so in comparison to *Mycosphaerella dearnessii*. There exists a potential for the involvement of toxins in the host/pathogen relationships examined which should be investigated.

Introduction

Although an extensive literature exists concerning the taxonomy of many of the needlecast and needle blight fungi (7,8,16), little information is available concerning the effect of these pathogens on the tissues of their respective hosts. Such research is vital to a comprehensive understanding of the host/pathogen relationship of the various needle-inhabiting fungi.

The following is a limited summary of published and unpublished histopathological research on three needlecast and two needle blight fungi on pines from the United States. Similarities and differences in the host tissue reactions produced by and characteristic morphological features of the individual pathogens are emphasized. Unfortunately, it is not possible to report these results from the same host pine nor for a particular pathogen on several host pines. Under these and other restraints, this paper will describe details of the host/pathogen relationships of the following: *Ploioderma hedgecockii* (Dearn.)Darker [=*Hypoderma hedgecockii* Dearn. (8)] and *Mycosphaerella dearnessii* Barr [=*Scirrhia acicola* (Dearn.)Siggers (11)] on *Pinus palustris* Mill. (longleaf pine), *Ploioderma lethale* (Dearn.)Darker [=*Hypoderma lethale* Dearn. (8)] and *Lophodermium australe* Dearn. on *Pinus taeda* L. (loblolly pine), and *Mycosphaerella pini* E. Rostrup apud Munk (11) [=*Dothistroma pini* Hulbary] on *Pinus ponderoa* Laws. (ponderosa pine) and *P. nigra* Arn.(Austrian pine).

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Host/Pathogen Relationships

Dothistroma pini Hulbary.— This pathogen is the causal agent of a needle blight on several species of *Pinus*. Taxonomically, there is a close relationship with the brown-spot needle blight pathogen, *M. dearnessii*, where differing color of conidia is a major distinguishing feature separating the two fungi. Peterson and Walla (19) published on the histology of *D. pini* and this discussion will deal with that work.

In needles of *P. nigra* collapse of mesophyll cells in symptomatic areas was an outstanding and common feature of affected tissue regions. The collapse was sudden, occurring in the presence of only three hypal strands developed from the initial penetrations of the pathogen. Initially, the hyphae were intercellular in the mesophyll. Hyphae were most common in the mesophyll, but were also observed in resin canals, endodermis, transfusion, and vascular tissues. Inter- and intracellular hyphae were present in the transfusion tissue. Epidermal or hypodermal cells apparently were not collapsed. A zone of stained intercellular material separated healthy and collapsed tissue regions.

In needles of *P. ponderosa* the effects of *D. pini* were somewhat similar to observations of *P. nigra*. The affected areas of needles were smaller than in *P. nigra* but did exhibit mesophyll collapse and hyphal presence. A zone of stained intercellular material between healthy and collapsed regions was not observed.

Illustrations in the work of Peterson and Walla indicate sparse hyphal presence in collapsed mesophyll, even in affected tissues subtending stromata. Another interesting feature was the apparent bending or crushing of the mesophyll under stromata, which in turn suggests a disruption or distortion of the hypodermal cells or layer normally found above the mesophyll. The presence of a toxin was not indicated by

Peterson and Walla, but Shain and Franich (21) presented evidence of a toxin involvement with *D. pini* and *P. radiata* D. Don.(Monterey pine).

Mycosphaerella dearnessii Barr.— This is the pathogen of the brown-spot needle blight of southern pines, which is a serious disease in young plantations and stands of natural reproduction of *P. palustris*. A recent paper outlined the histology of this host/pathogen relationship (13).

Cellular collapse in the mesophyll of symptomatic needle tissue was the most conspicuous tissue abnormality observed (fig. 1A-C). Normally, all mesophyll cells were uniformly collapsed in symptomatic areas. Hyphae were rare or difficult to observe in the collapsed tissues except subtending stromata (fig. 1B,C). This lack of hyphal presence gave the collapsed areas an empty appearance (fig. 1B,C). Abnormal host tissue and hyphae of the pathogen were limited to the symptomatic area and to tissues outward from the endodermis. Rarely were endodermal cells colonized or invaded by hyphae. There was a distinct separation of collapsed and unaffected tissues (fig. 1A). Limited presence of staining intercellular material was observed separating areas of collapsed and unaffected tissues. Epidermal and hypodermal cells were not invaded by hyphae, although at stromata these cells often were displaced. The hypodermis often was displaced inward under stromata causing a bending or crushing of underlying mesophyll (fig. 1C.) The amount of host tissue damage in relation to the sparse hyphal presence of *M. dearnessii* strongly indicated the potential of a toxin involvement in the host/pathogen relationship. This theory needs further investigation.

Ploioderma hedgcockii (Dearn.) Darker.— This is one of the tar-spot fungi causing needlecast of two- and three-needled pines (1, 9, 10, 12). Other than a few brief references in the literature, little is known of this pathogen. A short report has been published (14) and a paper has been submitted on the *P. hedgcockii/ P.palustris* relationship (15).

The effect of the pathogen on the host was restricted to the symptomatic areas of the needles. The most obvious tissue abnormality was a cellular collapse evident in the mesophyll tissues (fig. 1D-F). The symptomatic areas did not exhibit uniform collapse of affected mesophyll. Frequently, non-collapsed and normal-appearing mesophyll cells were observed in close association (fig. 1E). The outer cell walls of the endodermis were the inward limit of host tissue abnormality. Rarely was the endodermis affected. There were profuse inter- and intracellular hyphae of the pathogen in the affected tissues of the symptomatic areas. The areas between collapsed cells often were completely packed with hyphae (fig. 1D-F). This was the most outstanding characteristic of this host/pathogen relationship. A conspicuous staining intercellular material was present at the rather sharp area of separation of normal and diseased mesophyll tissues. Hyphae or tissue abnormalities were not observed beyond the lateral symptom boundaries. Endodermal, hypodermal, epidermal, or transfusion/vascular tissues were not colonized by the pathogen. Resin ducts exhibited necrosis and hyphal colonization. Stromata were seated on the hypodermis and did not appear to crush or displace the underlying hypodermal tissue (fig. 1F). Host tissue damage only in close association with hyphae of the pathogen does not indicate potential for a strong involvement of a toxin in this host/pathogen relationship.

Ploioderma lethale (Dearn.) Darker.— This is a common needlecast fungus affecting several species of pine. Boyce (3,4) and Campbell (5) noted outbreaks of this pathogen. Czabator et al (6) reported the presence of *P. lethale* as an associate in a widespread needle blight of southern pines.

The symptomatic areas of host needles exhibited cellular collapse of the mesophyll (fig. 2A,B,D). There appeared to be a sharp differentiation between noncollapsed and collapsed mesophyll tissue, and a definite constriction of the symptomatic area adjacent to the normal green needle tissue (fig. 2A). This constriction was apparently due to a breakdown of the walls of the collapsed mesophyll and collapse of the underlying endodermis. The vascular tissue of the symptomatic area appeared non-functional. This vascular condition extended into the normal-appearing tissue adjacent to the symptom boundary, often to a depth of >75µm. Other than the abnormality in the vascular system, tissue abnormalities were limited to the symptomatic areas. No staining intercellular material was observed at the symptom boundaries. The mesophyll and hypodermal layers were pushed inward under stromata (fig. 2D). Sparse inter- and intracellular hyphae were present in the affected tissues (fig. 2B). Hyphae were difficult to observe in transfusion and vascular tissues of symptomatic areas. Hyphae were frequently observed beyond the boundaries of the symptomatic areas, often >200µm into normal-appearing mesophyll. This hyphal presence was not associated with host cell abnormalities (fig. 2C). No other needle tissues beyond the symptom boundaries appeared to be colonized by the pathogen. Toxin involvement in the *P. lethale/P. taeda* relation requires investigation. The death of vascular tissue in the absence or scarcity of hyphae suggests the potential role of a toxin.

Lophodermium australe Dearn.— This fungus has a confusing ecology. Although reported from several pine species (1,2,16,17), the pathogenicity of this fungus is uncertain. It has been considered primarily a saprophyte on needles dead from other causes (17) while other reports strongly suggest pathenogenicity

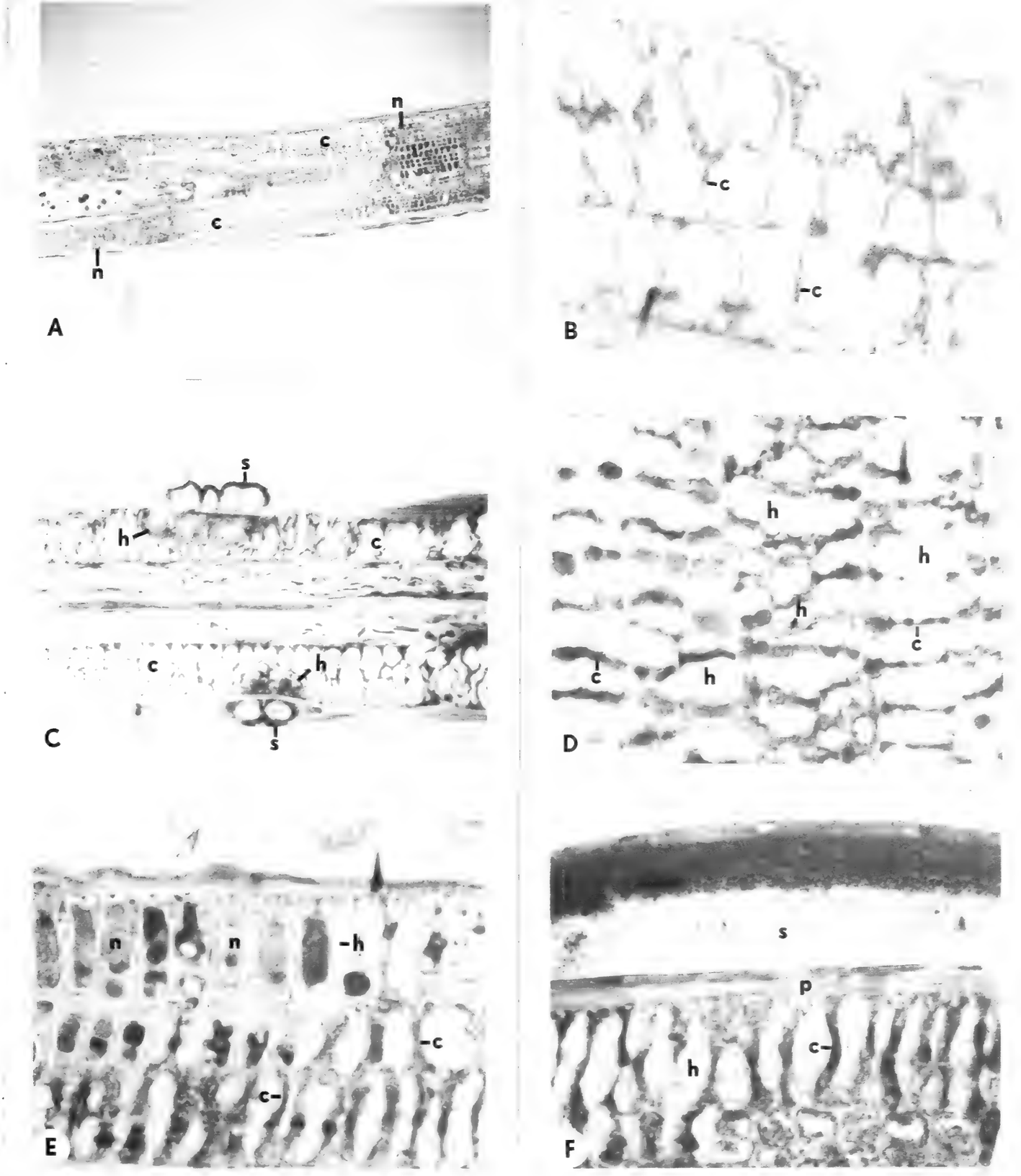

Figure 1.—A - C = *Mycosphaerella dearnessii*; D - F = *Ploiodrema hedgcockii*. All illustrations are from longitudinal sections. A) Section thru the mesophyll tissue. Note the noncollapsed and collapsed mesophyll cells, and the rather sharp separation of the two tissue types (X40); B) Collapsed mesophyll area exhibiting empty appearence due to lack of hyphae (X40); C) Stroma-bearing tissue exhibiting concentration of hyphae beneath stromata. Note bending of mesophyll by stroma (X75); D) Hyphae packed into intercellular spaces caused by collapse of mesophyll cells (X470); E) Noncollapsed and collapsed cells in close association. Note large intercellular hypha (X470); F) Undisturbed hypodermis under stroma (X470).

c = collapsed mesophyll cell(s)
n = non-collapsed mesophyll cell(s)
v = transfusion/vascular tissue
h = hypha(e)
s = stroma
p = hypodermis

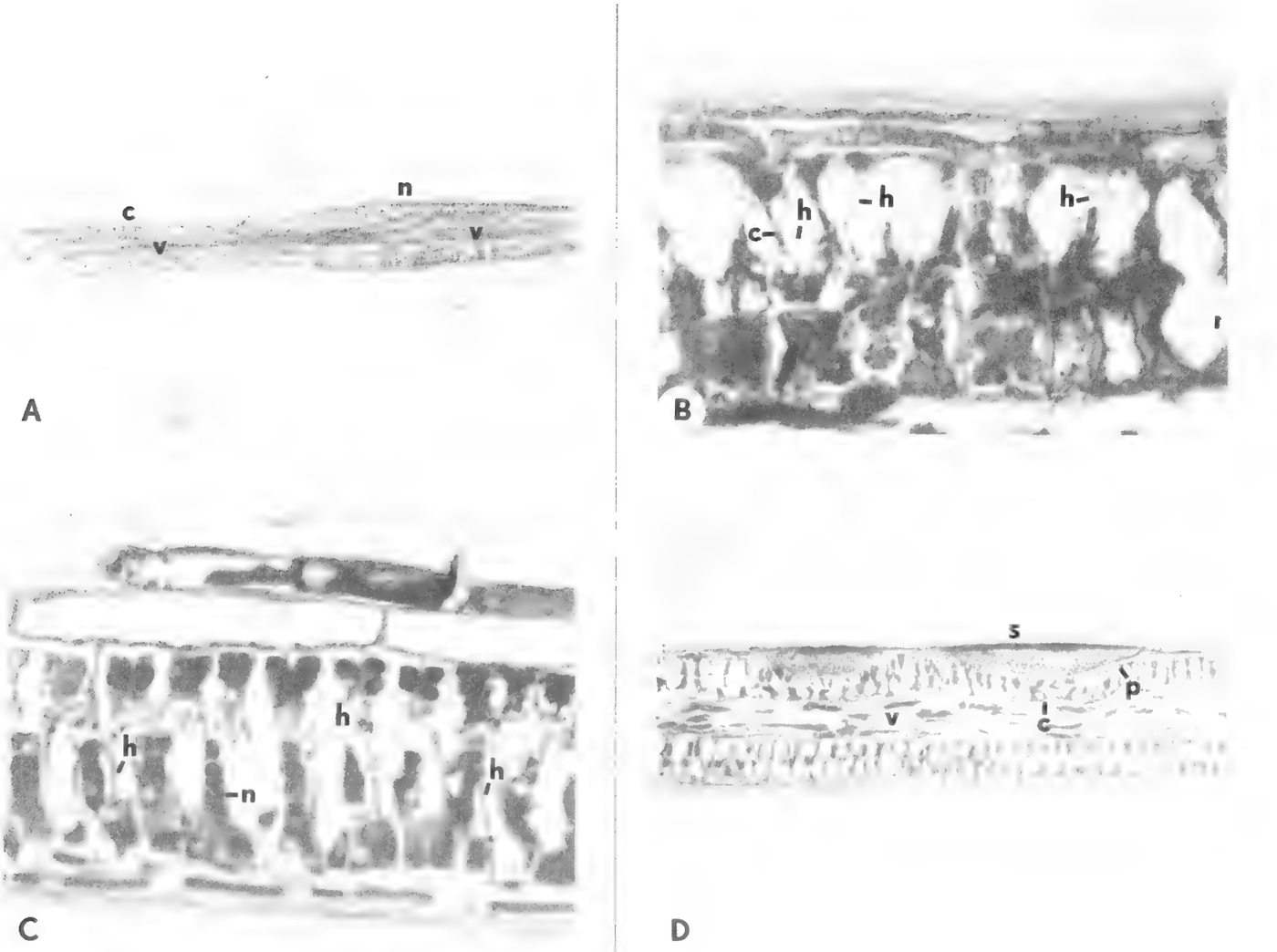

Figure 2.—A - D = *Ploioderma lethale*. All illustrations are from longitudinal sections. A) Constriction of needle at symptomatic area exhibiting collapsed mesophyll cells. Note distinct separation of symptomatic area from normal tissue (X40); B) Hyphae in intercellular spaces created by cell collapse in mesophyll (X470); C) Hyphae in normal- appearing mesophyll adjacent to symptomatic area (X470); D) Bending of hypodermis and mesophyll under stromata (X75).

c = collapsed mesophyll cell(s)
n = non-collapsed mesophyll cell(s)
v = transfusion/vascular tissue
h = hypha(e)
s = stroma
p = hypodermis

(2,20). The author believes this fungus to be pathogenic, particularly on living (green) needle tissue stressed in some manner

Determinations for *L. australe* were made from dead needle tissue adjacent to green tissue, as this was considered a typical symptom expression for this pathogen. To insure the correct material was utilized, samples with typical stromata (hysterotheca) of the pathogen were preferred, but non-stromatic samples were taken from fascicles with stroma-bearing needles present. From this material, the principal host abnormalities exhibited were collapse of the mesophyll tissues and the non-functional appearance of the transfusion/vascular tissues (fig. 3A,C,D). Endodermal tissues were difficult to determine, and often appeared absent. No constriction of the needles was evident in symptomatic areas. A rather distinct separation of diseased and normal-appearing (green) tissue was exhibited at the symptom boundary, but no staining intercellular material was observed (fig. 3B). Intra- and/or intercellular hyphae were present in collapsed mesophyll areas, endodermis, and the transfusion/vascular tissues (fig. 3C). Hyphal presence was limited in comparison to the amount of host cell damage (fig. 3A-C). Hyphae were observed in normal

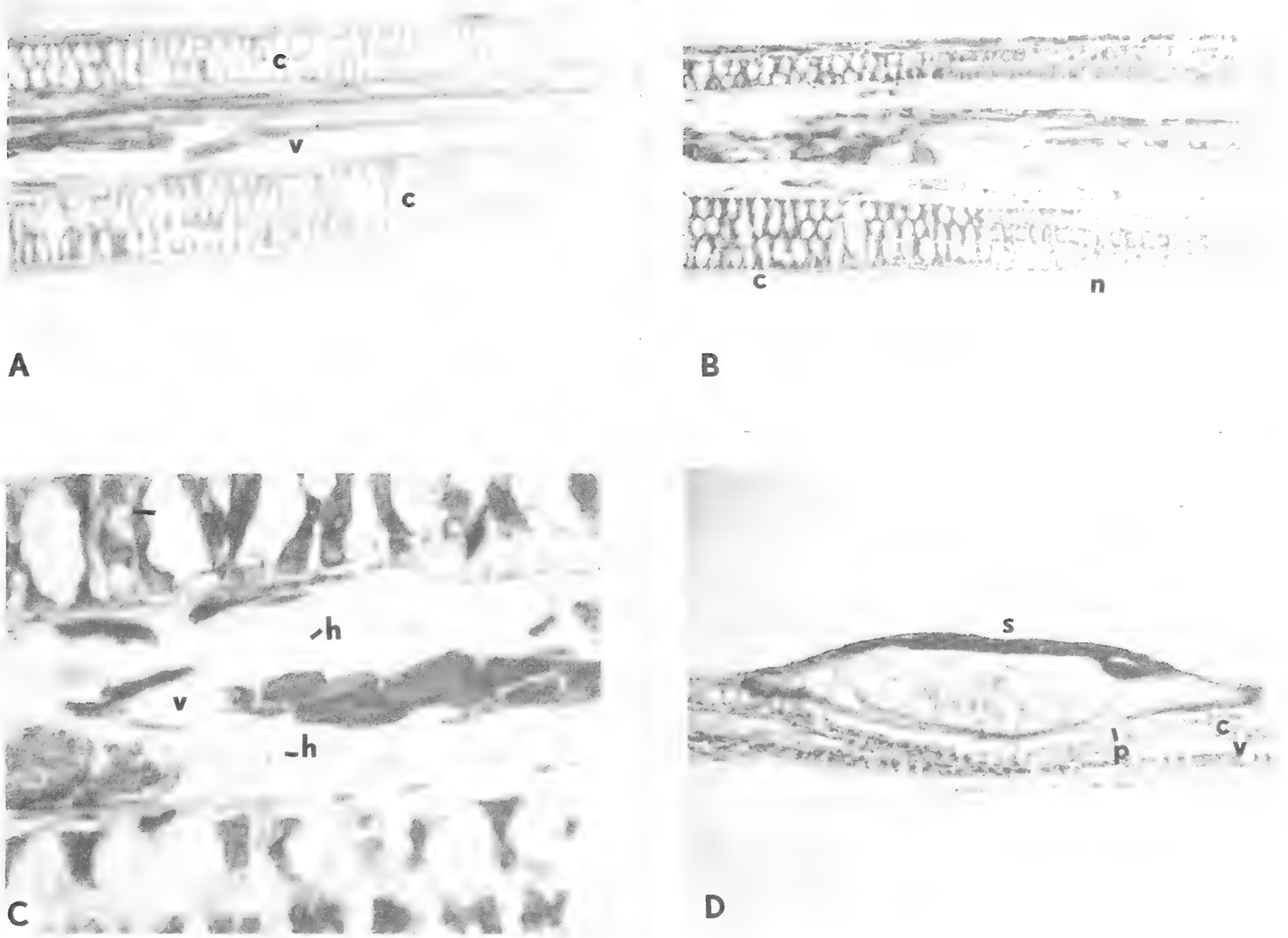

Figure 3.—A - D = *Lophodermium australe*. All illustrations are from longitudinal sections. A) Collapsed mesophyll and non-functional transfusion/vascular tissue. Note lack of hyphae and empty appearence of affected mesophyll area (X75); B) Definitive separation of diseased and normal-appearing tissues at symptom boundary (X75); C) Hyphae in transfusion/vascular tissue in symptomatic area (X470); D) Bending and crushing of host tissues under stroma (X75).

c = collapsed mesophyll cell(s)
n = non-collapsed mesophyll cell(s)
v = transfusion/vascular tissue
h = hypha(e)
s = stroma
p = hypodermis

appearing mesophyll in green tissue adjacent to symptom boundaries. Stroma development resulted in disruption, bending or crushing of underlying hypodermal, mesophyll, endodermal, and transfusion/vascular tissues (fig. 3D). The potential of a toxin in the relationship of *L. australe* and its pine hosts requires investigation.

Discussion

In observations of the various host/pathogen relationships, the one tissue abnormality common to all was the collapse of mesophyll tissue in affected areas of symptomatic needles. This collapse was, in most instances, complete throughout the symptom areas with the exception of *P. hedgcockii*. Also, with the exception of the latter, the mesophyll cell collapse was not associated with a profusion of hyphae of the individual pathogens. This was particularly true with *P. palustris* on which both *P. hedgcockii* and *M. dearnessii* were studied. The former produced profuse amounts of hyphae in affected mesophyll while hyphae of the latter were absent or very difficult to observe. *Lophodermium australe* and *P. lethale* on *P. taeda* produced similar host effects, but differed in hyphal presence in affected tissues. Regardless of the abundance of hyphae of the needle pathogens examined, the cellular collapse in the mesophyll was similar

with all pathogens involved in these studies. The causative factor of the mesophyll cell collapse requires investigation. A toxin, either host- or pathogen produced, is suggested as a potential factor in the needle fungi/host relationships observed.

The colonization of host tissues other than the mesophyll occurred with *D. pini, L. australe,* and *P. lethale,* while *P. hedgcockii* and *M. dearnessii* were limited primarily to the mesophyll tissues within symptom boundaries. The colonization of transfusion/vascular tissues and the advance of hyphae beyond symptom boundaries by *D. pini, L. australe,* and *P. lethale* indicate a more aggressive relationship with their respective hosts. If, as suggested, the colonized transfusion/vascular tissues become non-functional, the physiological future of such needles would be in doubt. This might explain the consistent occurrence of these pathogens on dead needle tissue, while *M. dearnessii* and *P. hedgcockii* often languish for considerable periods of time on green (living) needles or needle tissue.

The bending of tissues subtending stromata of certain of the studied pathogens has been documented or illustrated (7,9,13,19). Although the degree to which inward bending of tissues varied per pathogen, all but one produced this effect. The exception, again, was *P. hedgcockii,* where no displacement inward of tissues subtending stromata was observed.

The presence or nonpresence of the intercellular staining material at symptom boundaries of certain of the pathogens was of considerable interest. What this material is or represents is not known at this time. Mitchell et al (18) and Williamson (22) reported a stationary interface in pine tissue infected by two species of *Lophodermella.* The interface described by them appears similar in appearance and location to the staining intercellular material observed in the present research. Thus, it is reasonable to assume a stationary interface occurs in host tissue infected with certain of the pathogens studied, particularly *P. hedgcockii* and *M. dearnessii.*

Literature Cited

1. Anon. 1960. Index of plant diseases in the United States. USDA Agric. Handb. 165.
2. Bega, R.V., Smith, R.S., Martinex, A.P., Davis, C. J. 1978. Severe damage to *Pinus radiata* and *P. pinaster* by *Diplodia pinea* and *Lophodermium* spp. on Molokai and Lanai in Hawaii. Plant Dis. Rep. 62:329-331.
3. Boyce, J. S. Jr. 1954. Hypoderma needle blight of Southern pines. J. For. 52:496-498.
4. Boyce, J.S., Jr. 1958. Needle cast of Southern pines. USDA Forest Serv. For. Pest Leaflet 28.
5. Campbell, W.A. 1949. Needle cast of Southern pines. Forest Farmer. 9:4,10.
6. Czabator, F.J., Staley, J.M., Snow, G.A. 1971. Extensive Southern pine needle blight during 1970-1971, and associated fungi. Plant Dis. Rep. 55:764-766.
7. Darker, G.D. 1932. The Hypodermataceae of conifers. Contr. Arnold Arbor. 1:1-131.
8. Darker, G.D. 1967. A revision of the genera of the Hypodermataceae. Can J. Bot. 45:1399-1444.
9. Dearness, J. 1926. New and noteworthy fungi — IV. Mycologia. 18:236-255.
10. Dearness, J. 1928. New and noteworthy fungi — V. Mycologia. 20:235-246.
11. Evans, H.C. 1984. The genus *Mycosphaerella* and its anamorphs *Cercoseptoria, Dothistroma,* and *Lecanosticta.* Comm. Mycol. Inst. Mycol. Papers No. 153.
12. Hedgcock, G.G. 1932. Notes on the distribution of some fungi associated with diseases of conifers. Plant Dis. Rep. 16:28-42.
13. Jewell, F.F. Sr. 1983. Histopathology of the brown-spot fungus on longleaf pine needles. Phytopathology. 73:854-858.
14. Jewell, F.F. Sr. 1987. Histopathology of *Ploioderma hedgcockii* on longleaf pine needles. (Abstr.) Phytopathology. 77:1718.
15. Jewell, F.F.Sr. 1989. Histology of longleaf pine needles infected by *Ploioderma hedgcockii* (Dearn.)Darker. Mns. submitted.
16. Minter, D.W. 1981. *Lophodermium* on pines. Comm. Mycol. Inst. Mycol. Papers No. 147.
17. Minter, D.W. 1986. Some members of the Rhytismataceae (Ascomycetes) on conifer needles from Central and North America. p. 71-106. In: G.W. Peterson (ed.) Recent Research on Needle Diseases. USDA Forest Serv. Gen. Tech. Rep. WO-50. 106 p.
18. Mitchell, C.P., Millar, C.S., Williamson, B. 1978. The biology of *Lophodermella conjuncta* Darker on Corsican pine needles. Eur. J. For. Path. 8:108-118.
19. Peterson, G.W., Walla, J.A. 1978. Development of *Dothistroma pini* upon and within needles of Austrian and ponderosa pines in Eastern Nebraska. Phytopathology. 68:1422-1430.

20. Roux, R.F., Lundquist, J.E. 1984. Needle disease found on pines in South Africa, caused by *Lophodermium australe, L. seditiosum,* and *L. indianum.* Plant Dis. 68:628.

21. Shain, L., Franich, R.A. 1981. Induction of *Dothistroma* blight symptoms with dothistromin. Physiol. Plant Pathol. 19:49-55.

22. Williamson, B., Mitchell, C.P., Millar, C.S. 1976. Histochemistry of Corsican pine needles infected by *Lophodermella sulcigena*(Rostr.)v. Hohn. Ann. Bot. 40:281-288.

Appressoria and Their Possible Use For Identification of the Rhytismataceae (Ascomycetes)[1,2]

B. R. Stephan[3] and M. Osorio[3,4]

Abstract.—Information on appressorium formation in species of the Rhytismataceae (Ascomycetes) is very scarce. Therefore, development and morphology were examined in *Cyclaneusma minus, Lirula macrospora, Lirula* sp., *Lophodermium arundinaceum, L. juniperinum, L. piceae, L. pini-excelsae, L. pinastri, L. seditiosum,* and *Meloderma desmazieresii*. All species except *C. minus* formed appressoria after 12-24 hours incubation. Sizes and forms were typical and specific for each species. Appressoria can therefore be useful additional morphological and taxonomical criteria for identification.

Introduction

Appressoria are described in many fungi of various ecological and phylogenetic groups. They are formed on germ tubes of ascospores and conidia or on vegetative hyphae in vivo and in vitro, mostly as a result of stimuli from mechanical contact with a substrate (3). The morphological characteristics of appressoria are very distinct, and have been used sometimes for taxonomical separation of fungal species, e.g. in *Colletotrichum* species (1, 8, 9, 10, 11, 12), in *Phyllachora* species (7) and in some species of powdery mildew (13, 14). There are only a few reports concerning appressorium formation in ascospores of species of the family Rhytismataceae (Ascomycetes), although earlier Darker (2) observed and illustrated appressoria in various species of this family.

In connection with studies on the biology of the needle endophyte, *Lophodermium piceae* (Fuckel) v. Höhnel, appressorium formation of several other species was investigated (5). The main results are summarized in the following. A more detailed article has been published elsewhere (6).

Materials and Methods

About 90 samples of 10 species were collected from different host plants and from various countries. With the exception of *Lophodermium arundinaceum* (Schrad.) Chev. from the grass *Dactylis glomerata* L., the other species were collected from conifer needles: *Cyclaneusma minus* (Butin) DiCosmo, Peredo & Minter, *Lirula macrospora* (Hartig) Darker, *Lirula* sp., *Lophodermium juniperinum* (Fr.) de Not., *Lophodermium piceae*, *Lophodermium pini-excelsae* Ahmad, *Lophodermium pinastri* (Schrad.) Chev., *Lophodermium seditiosum* Minter, Staley & Millar, and *Meloderma desmazieresii* (Duby) Darker.

For germination trials only fresh and mature ascomata were used. After collection they were stored in a refrigerator at -5 C. The detailed procedure for studying ascospore germination has been described previously (6). Ascospore germination and appressorium formation were observed in sterile distilled water for about 2 weeks.

Results

Germination of ascospores was observed in all of the ten species of Rhytismataceae, although not all samples germinated. Only fresh and mature ascospore material germinated. With few exceptions, ascospores from older ascomata or from material stored for long time in a refrigerator did not germinate. There were also significant differences in the germination percentage among species and samples. Unfavorable conditions seemed to influence the germination process.

Ascospore germination started after 8-12 hours and ended with the formation of appressoria. A comparison of the behavior of the germinating ascospores showed clear and specific peculiarities among the fungal species studied.

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The ascospores of all species germinated with more or less long hyphae. The length of these connecting hyphae between ascospore and appressorium was typical for each fungus. The measurements of length and width together with the position of the connecting hyphae are listed in table 1.

Nine of the ten fungal species formed appressoria under the given environmental conditions. The only exception was *Cyclaneusma minus*, which germinated without forming appressoria. Within a species the appressoria were very uniform, even in specimens of the same species but from different localities. Peculiarities of the appressoria as to form, size and number are given in table 1. Drawings of the appressoria are shown in figure 1.

Summarizing, one can state that the form of the appressorium can be simple, more or less round, branched or variable in shape. But despite the variation observed, a distinction among species on basis of appressoria characteristics is possible. Each fungal species examined had its specific and constant type of appressorium. In this connection it is of great interest that even ascospore material of the same species but from different hosts formed identical appressoria. This is illustrated by figure 1 B, G, H and I, where examples of *Lophodermium piceae* are shown from *Picea mariana* (Mill.) B.S.P., *P. abies* (L.) Karst., *Abies alba* Mill., and *P. sitchensis* (Bong.) Carr. X *P. omorika* (Pancic) Purkyne, respectively.

Also interesting is that *Lophodermium pinastri* rarely germinated and thus appressoria also were rare. But when appressoria were formed, they resembled those of *L. seditiosum*. Both species are closely related, and were not distinguished until Minter et al. (4) separated the *L. pinastri*-complex into the three species, *L. pinastri, L. seditiosum* and *L. conigenum* (Brunaud) Hilitzer. The close relationship of the first two species was confirmed by the appressoria studies.

Conclusion

The results presented here demonstrate, together with the evaluation of drawings in the paper of Darker (2), that shape and size of the appressoria are constant within and differ among the species examined. They offer possibilities for use as additional taxonomical characteristics for the identification of members of the family Rhytismataceae. If fresh and mature ascomata material is available, the examination of appressoria is a quick and easy method. Future studies should include further species, particularly species from hosts other than conifers.

Literature Cited

1. Arx, J.A.. v. 1957. Die Arten der Gattung *Colletotrichum* Cda. Phytopath. Z. 29:413-468.
2. Darker, G.D. 1932. The Hypodermataceae of conifers. Contrib. Arnold Arboretum 1:1-131.
3. Emmett, R.W., Parbery, D.G. 1975. Appressoria. Ann. Rev. Phytopath. 13:147-167.
4. Minter, D.W., Staley, J.M., Millar, C.S. 1978. Four species of *Lophodermium* on *Pinus sylvestris*. Trans. Brit. Mycol. Soc. 71:295-301.
5. Osorio, M. 1989. Zur Biologie des in Fichtennadeln vorkommenden Pilzes *Lophodermium piceae* (Fuckel) v. Höhnel. Dissertation. Georg-August-Universität, Göttingen. 175 p.
6. Osorio, M., Stephan, B.R. 1989. Ascospore germination and appressorium formation in vitro of some species of the Rhytismataceae. Mycological Research 92 (in press).
7. Parbery, D.G. 1963. Studies on graminicolous species of *Phyllachora* Fckl. I. Ascospores - their liberation and germination. Austral. J. Bot. 11:117-130.
8. Stephan, B.R. 1967. Untersuchungen über die Variabilität bei *Colletotrichum gloeosporioides* Penzig in Verbindung mit Heterokaryose. I. und II. Zentralblatt f. Bakteriologie, Parasitenkunde, Infektionskrankheiten u. Hygiene, II. 121:41-57, 58-72.
9. Stephan, B.R. 1973. *Colletotrichum crassipes* (Speg.) v. Arx als Krankheitserreger an *Tillandsia*-Arten. Nachrichtenblatt d. Deutschen Pflanzenschutzdienstes (Braunschweig) 25:82-84.
10. Sutton, B.C. 1962. *Colletotrichum dematium* (Pers. ex Fr.,) Grove and *C. trichellum* (Fr. ex Fr.) Duke. Trans. Brit. Mycol. Soc. 45:222-232.
11. Sutton, B.C. 1968. The appressoria of *Colletotrichum graminicola* and *C. falcatum*. Can. J. Bot. 46:873-876.
12. Sutton, B.C. 1980. The Coelomycetes. CAB International Mycological Institute. Kew, U.K. 696 p.
13. Zaracovitis, C. 1965. Attempts to identify powdery mildew fungi by conidial characters. Trans. Brit. Mycol. Soc. 48:553-558.
14. Zaracovitis, C. 1966. The germination in vitro of conidia of powdery mildew fungi. p. 273-286 In: M.F. Madelin (ed.), The Fungus Spore, Butterworths, London.

Table 1. Form, size and other details of appressoria in some species of the family Rhytismataceae (from OSORIO and STEPHAN, 1989)

Species	Connecting Hypha between Ascospore and Appressorium			Appressorium			
	Length (µm)	Width (µm)	Site	Form	Length (µm)	Width (µm)	Number per Ascospore
Cyclaneusma minus	Germinated, but did not form appressoria.						
Lirula macrospora	(15.0-)60.0-110.0(-120.0)	2.0(-2.4)	lateral to subapical	irregular with deeply lobed edges	(12.0-)14.0-22.0(-24.0)	4.0-6.0	1
Lirula sp.	(4.0-) 7.2-12.0(-17.6)	1.6(-2.4)	lateral to subapical	triangular to bell-shaped	(6.4-) 8.0- 8.8(-10.4)	(4.8-)5.6-8.0(-8.8)	1 (except.2)
Lophodermium arundinaceum	(12.8-)24.0-32.0(-43.2)	1.6	lateral to subapical (except. apical)	irregular, clavate with crenate or deeply lobed edges	(16.0-)16.8-24.8(-26.4)	3.2-4.0	1
Lophodermium juniperinum	(4.0-)12.0-32.0(-56.0)	1.6	lateral to subapical	irregular, clavate with crenate or deeply lobed edges	(5.6-) 8.0-10.4(-11.2)	2.4-3.2	1 (except.2 to 3)
Lophodermium piceae	(3.2-) 4.8-12.0(-20.0)	(1.2-)1.6	lateral to subapical (except. apical)	bean- to kidney-shaped	(7.2-) 8.0- 8.8(- 9.6)	4.8-5.6	1 (except.2)
Lophodermium pinastri	Germination very seldom			If appressoria are developed, their size and form resemble to L. seditiosum			
Lophodermium pini-excelsae	0 (-2.4)	0 (-2.4)	lateral, subapical or apical	fusiform to clavate	(11.2-)12.0-17.6(-20.0)	3.2-4.0	1
Lophodermium seditiosum	0-10.0	1.6-2.4	lateral, subapical or apical	fusiform to clavate sometimes slightly lobed	(10.0-)12.0-18.0(-20.0)	(4.0-)5.0-8.0(-11)	1 (except.2)
Meloderma desmazieresii	0 (- 9.6)	0 (-3.2)	lateral to subapical	clavate, with a bend basis	(20.0-)22.0-28.0(-32.0)	4.0-4.8(5.6)	1

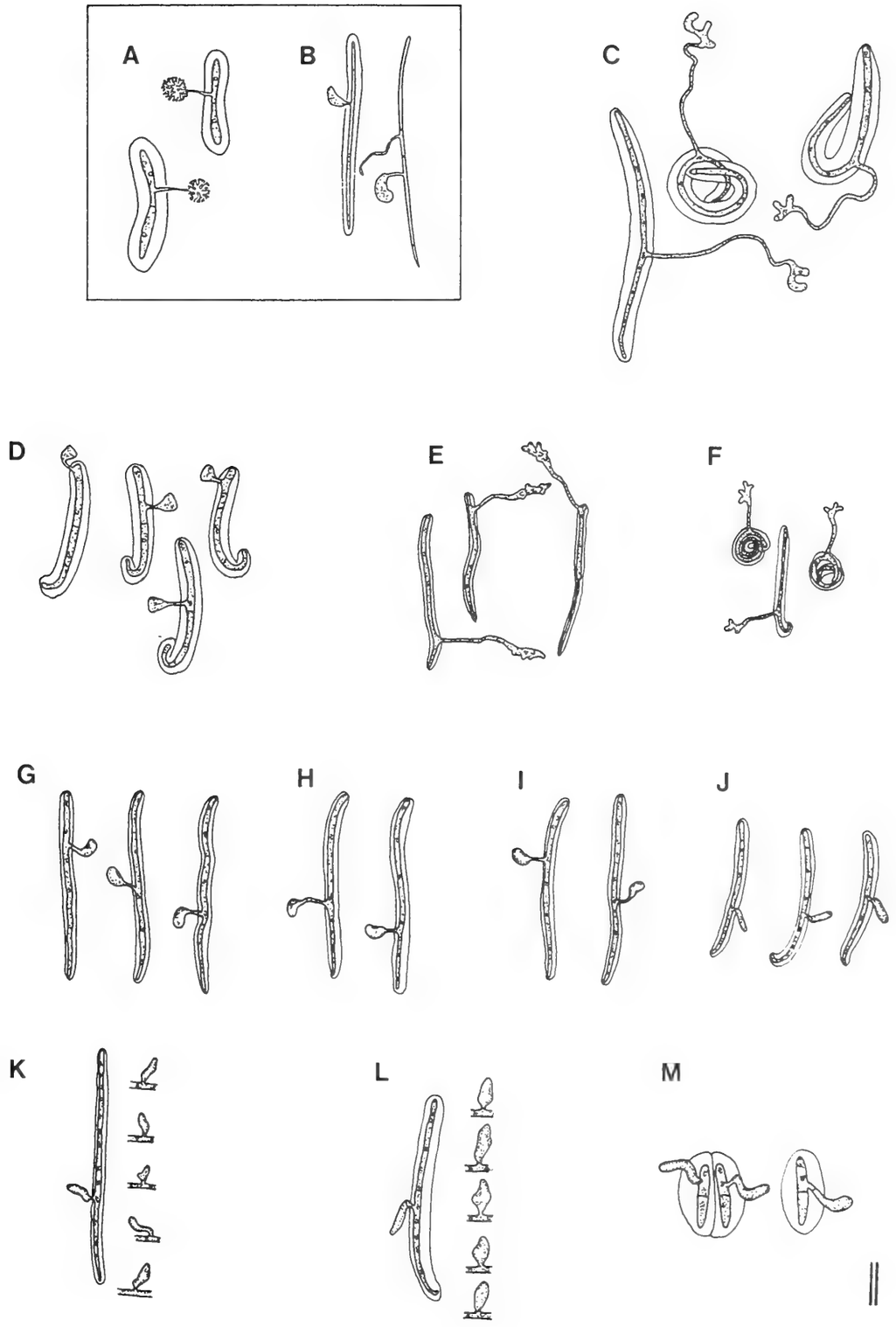

Figure 1.—Germinating ascospores and form of the appressoria in some species of the Rhytismataceae. A. *Bifusella faullii* on *Abies balsamea* (L.)Mill. B. *Lophodermium piceae* on *Picea mariana* (A and B, from Darker, 1932). C. *Lirula macrospora* on *Picea glauca* (Moench) Voss. D. *Lirula* sp. on *Picea abies* (L.) Karst. E. *Lophodermium arundinaceum* on *Dactylis glomerata* L. F. *Lophodermium juniperinum* on *Juniperus chinensis* L. cv. *Pfitzeriana*. G. *Lophodermium piceae* on *Picea abies*. H. *Lophodermium piceae* on *Abies alba*. I. *Lophodermium piceae* on *Picea sitchensis* x *omorika*. J. *Lophodermium pini-excelsae* on *Pinus strobus* L. K. *Lophodermium pinastri* on *Pinus sylvestris* L. L. *Lophodermium seditiosum* on *Pinus sylvestris*. M. *Meloderma desmazieresii* on *Pinus monticola* Dougl. ex D. Don. (Bar equals 20 µm).

Thoughts on Conifer Needlecasts[1,2]

W. Merrill[3]

Abstract.—Needle blights are caused by various Ascomycetes and Fungi Imperfecti which have repeating cycles of asexual spores, relatively short incubation periods and short generation times. Thus, needle blights typically are compound interest diseases capable of explosive epidemics. In contrast, needlecasts are caused principally by members of the Rhytismatales with one infection cycle of ascospores per year and with prolonged incubation periods and prolonged periods from infectious spore to infectious spore. Thus, needlecasts are simple interest diseases characterized by tardive epidemics. Major deficiencies in our knowledge of needlecasts are discussed.

Reflecting upon the presentations and discussions of this working party conference, I was struck by two thoughts: first, the confusion over terminology, and second, our limited knowledge base, that is, how little we know but how much we assume.

Terminology

Needle blights:—Discussion focused on use of the terms "needlecast" and "needle blight". "Blight" is a term commonly used to denote a sudden and rapid death of foliage, in most cases applied to those diseases where the pathogen attacks the foliage itself (5). Potato late blight, caused by *Phytophthora infestans* (Mont.) de Bary, is a classic example. The incubation period (time from infection until symptom development) requires as little as 2.5 to 3 days, and the repeating cycle from infectious spore to infectious spore may be as short as 4 to 5 days. Thus, a "typical" blight is a compound interest disease, with multiple infection cycles per year often resulting in very high r values and therefore the development of explosive epidemics (10).

Needle blights are caused by a variety of pathogenic Ascomycetes and Fungi Imperfecti. The pathogens often infect needles of any age at any time of the year. Such pathogens typically have relatively short incubation periods and short cycles from infectious spore to infectious spore, and are compound interest diseases. The repeating cycles usually involve asexual spores. Dothistroma and brown spot needle blights are perhaps the best known examples. The so-called "larch needlecast", caused by *Mycosphaer*ella *laricina* (Hart.) Neg., is a needle blight. In this respect, Cyclaneusma needlecast, with a pathogen able to infect needles of any age at any time of the year, is more similar to the needle blights than to other needlecasts.

Needlecasts:—Many diseases result in the casting or shedding of conifer needles. Hartig (4) noted the "blighting" of needles caused by a "needlecast" (Nadelschütte). Hubert (6) separated the needlecasts from the needle blights on the basis of pathogen taxonomy; needlecasts were diseases caused by the Hysteriales. Rhabdocline needlecast, caused by a member of the Phacidiales, was called a needle blight. In his first edition, Boyce (2) used the terminology of Hartig, but in his second edition (3) separated the two type-diseases. For the past half century "needlecast" has been applied primarily to diseases caused by fungi in the Hysteriales, Hysteriaceae, Hypodermataceae, Phacidiaceae, Phacidiales, Rhytismataceae or Rhytismatales, depending upon the taxonomic scheme in vogue at the time. Ainsworth & Bisby's **Dictionary of the Fungi** (5) defines the term as, "(of conifers), loss of leaves caused by spp. of *Hypoderma, Lophodermium, Rhabdocline*, or other Rhytismatales." I urge that the term "needlecast" be reserved for such diseases, with few exceptions. Unfortunately, there are exceptions to most statements made about the needlecasts. In separating a needlecast from a needle blight, however, the symptoms and the taxonomy of the pathogen are less distinctive than the disease cycles and mode of epidemic development.Needlecasts are simple interest diseases. That is, there is one infection cycle per year, and the asexual spores, if produced by the pathogen, apparently function only as spermatia (exception: Rhizosphaera kalkhoffii Bubak produces only

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pycnidiospores). Most needlecast pathogens infect the succulent first-year needles during shoot elongation (exceptions: needlecasts caused by *Lophodermium* seditiosum Minter, Staley & Millar and *Cyclaneusma* minus (Butin) DiCosmo, Peredo & Minter)). Most of the pathogens have incubation periods requiring from 4 to 11 months. The complete disease cycle, from infectious spore to infectious spore, is lengthy, requiring 1 year for needlecasts caused by *Rhabdocline* spp., *L.* seditiosum and Ploioderma lethale (Dearn.) Darker, 2 years for needlecast of fir caused by *Isthmiella* faullii (Darker) Darker, and perhaps 3 or 4 years for *Lirula* macrospora (Hartig) Darker needlecast of spruces (9).

Because the pathogens lack asexual repeating cycles, needlecasts are simple interest diseases in any given year. Therefore, needlecast epidemics are not explosive, but tardive (10). On the other hand, for those needlecasts where the pathogen sporulates while the needles are still attached to the twigs and many of the spores thus are easily trapped by the host's developing young foliage, such as Rhabdocline needlecast, epidemic development may appear to have developed explosively following a year when climate was especially favorable for sporulation and infection, and when those conditions coincided with the period of maximum host susceptibility. But there are no repeating cycles of infection within a single growing season.

Knowledge Base

In spite of their common and widespread occurrence, very few of the needlecasts have been investigated. Koch's Posulates have been completed for only about a half dozen needlecast fungi. In part this is due to the prolonged incubation periods and in part to the fact that infectious ascospores do not form in culture (exception: *Cyclaneusma minus*) and are formed only once a year on infected needles. Needlecasts are difficult and time-consuming diseases to study. Thus we are forced to make assumptions based on a paucity of information or worse, casual observations.

For example, it is widely held that *Lophodermium nitens* Darker is a saprophyte because it produces hysterothecia only on fallen needles (8). Yet no one knows when these needles become infected or infested. Ascocarps of *L. nitens* often are present in late May or early June on needles of *Pinus strobus* L. lying on the top of the duff, needles that obviously fell the previous fall. If hysterothecia bearing spores are present in the spring, when did the needles on the duff become infected? Species of *Leptostroma* have been recovered from green needles and have been assumed to be nonpathogenic endophytes (1). Could these be the asexual stages of needlecast fungi which produce ascocarps only after the needles have dropped to the duff?

Many other basic questions remain unanswered. We know little about the precise environmental requirements for sporulation and infection by most of these fungi. We know little about the pathological anatomy of infected tissues, although some advances have been made for a few of the diseases (7). We know little of the effects of age of host tissues on infection; do many needlecast fungi infect juvenile needles because such needles have thin cuticles, or because they lack toxic extractives? Some needlecast fungi, such as *Lirula macrospora*, appear to have a variable length of life cycle. Is this true, and if so, why? Or is this due to the fact that two or more species are currently grouped as a single species? Needlecasts often are common and severe on understory reproduction. What effects do they have on growth of infected seedlings and saplings? What effects do they have on re-establishment of harvested stands?

The needlecasts are an intriguing group of diseases about which little is known, other than the names of the associated fungi. They deserve far more attention than they are likely to receive, given the difficulties of working with them!

Literature Cited

1. Carroll, G.C., Carroll, F.E. 1978. Studies on the incidence of coniferous needle endophytes in the Pacific Northwest. Can. J. Bot. 56:3034-3043.
2. Boyce, J.S. 1938. Forest Pathology, 1st ed. McGraw-Hill, New York. 600 p.
3. Boyce, J.S. 1948. Forest Pathology, 2nd ed. McGraw-Hill, New York. 550 p.
4. Hartig, R. 1874. Wichtige Krankheiten der Waldbäume. Beiträge zur Mycologie und Phytopathologie für Botaniker und Forstmänner. J. Springer, Berlin. 124 p.
5. Hawksworth, D.L., Sutton, B.C., Ainsworth, G.C. 1983. Ainsworth & Bisby's Dictionary of the Fungi, 7th ed. Commonwealth Mycological Institute, Kew, England. 445 p.
6. Hubert, E.E. 1931. An Outline of Forest Pathology. J. Wiley & Sons, New York. 543 p.
7. Jewell, F.F., Sr. 1990. Comparative histopathology of pine tissues infected by needlecast and needle blight fungi. p. 101-107. In: W. Merrill and M. Ostry (eds.). Recent Research on Foliage Diseases, USDA For. Serv. Gen. Tech. Rep. WO-56, 145 p.

8. Minter, D.W. 1981. *Lophodermium* on pines. Commonwealth Mycological Inst. Mycologial Papers 147, 54 p.

9. Walla, J. A. 1986. L*irula macrospora* on spruce in North Dakota: occurrence, symptoms, and spore release. p. 56-59. In: G.C. Peterson (ed.) Recent Research on Conifer Needle Diseases. USDA For. Serv. Gen. Tech. Rep. WO-50, 106 p.

10. Zadoks, J.C., Schein, R.D. 1979. Epidemiology and Plant Disease Management. Oxford University Press, Oxford. 427 p.

Pleuroceras pseudoplatani, Cause of Leaf Blotch Disease on Maple[1]

Alfred Wulf[2]

Abstract.—In 1986 and 1987 a leaf blotch disease of maple (*Acer pseudoplatanus*) caused by *Pleuroceras pseudoplatani* occurred commonly in Central Europe, providing a good opportunity for research on this seldom mentioned leaf disorder. In addition to a general description, development of symptoms and epidemiology are discussed. The perfect form, the newly found imperfect form, and the taxonomy of the causal fungus are described. The pure culture of the fungus is described and aspects of its possible control are discussed.

Introduction

During the summer of 1986 and 1987, a leaf blotch disease of maple (*Acer pseudoplatanus* L.) was observed on various sites in West Germany and neighboring countries, the symptoms of which were first described as and seen to be caused by a fungus by von Tubeuf in 1930 (8). The rather late description and the few existing reports (2, 3) can be explained by the fact that no fructifications of the fungus during its parasitic phase were known, preventing an identification. Therefore, to now, early diagnosis of the disease was dependent on the more or less characteristic symptoms. Since a microconidial form of the fungus was recently found which develops on the leaf stems and veins during the growing season, a positive identification is now possible while the disease is still developing. This possibility of reliable identification and a widespread occurrence of leaf blotch in the past few years prompted more research into the symptoms and the processes of infection and disease development.

Symptoms

With careful examination, the first inconspicuous infection points can be found on the under-surface of the leaf in early June. Usually, a larger leaf vein starts to turn black at the junction with a smaller vein. From this point, blackening spreads to adjoining veins and to the leaf tissues surrounding them. These spots typically develop an elliptical form along the leaf veins, visibly distinct from the green leaf tissue by August at the latest and reaching a maximum diameter of 3 cm (fig. 1). On the upper leaf surface, the spots appear brown with a darker edge which disappears with advanced development because of extension into the intercostal areas, giving way to an overall grey-brown tone of the spots. The center of the spot is reddish-brown, dry and brittle, and facilitates the identification of the initial infection center.

The blackened leaf veins on the leaf under-surfaces are clearly visible symptoms of the leaf blotch disease. With progressive development, these veins turn different shades of brown. In late summer, a partial wilt can sometimes be observed on leaves with large blotches, accompanied by shrinkage, crumpling and a yellow color above the affected tissues, leading to the impression of early autumnal changes. Severely affected leaves also become detached more easily and fall prematurely.

The development of all leaf blotches is more or less uniform, especially because of the absence of new infections during the course of the growing season. Therefore, all infections must occur within a relatively short period of time. This temporal restriction probably is due to the disposition of the unfolding leaves and to the influence of weather conditions on spore development and dissemination.

It is clear that fungal diseases originating from fruiting bodies on fallen leaves affect lower parts of the tree more severely. Examples are to be found for several tree diseases, especially for leaf disorders of maple, e.g., white spotting by *Cristulariella depraedans* (Cooke) Höhn. (4) and tar spot by *Rhytisma acerinum* (Pers. ex St. Am.) Fr. (6). It was assumed that the fungus causing leaf blotch of maple infects lower branches more frequently than higher branches, especially since the infections seem to be caused

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solely by ascospores from fallen, overwintered leaves. This assumption was substantiated by observations and evaluations on a number of trees. In the lower branches near the ground more than 80 % of the leaves were infected, in contrast to only 20 % in the upper branches.

Teleomorph of the Causal Fungus

The perithecia described first by von Tubeuf (8) were found within the original blotches after natural overwintering of infected maple leaves. At the beginning of April, most of the still easily visible and dark blotches showed developing perithecia, seen as small intercostal bulges. Ostioles extending above the leaf surface were found on only 10% of the leaves at this time, containing the first mature ascospores. By mid-May, 60% of the leaves had completely-formed fruiting bodies with mature ascospores, 30% had developing perithecia and 10% lacked perithecia in spite of typical blotches. Consequently, spore maturity coincides nicely with the presumed period of infection at the end of May.

The evaluation of infected leaves yielded 36 to 128 perithecia per square centimeter; the average was 80. This corresponds with von Tubeuf's report (8) of one perithecium per square millimeter. The fruiting bodies were more frequent near the edges of the blotch than in the center. Independent of which leaf side faced the ground, approximately 80% of the perithecial necks protruded through the upper leaf surface. A typical perithecium has a flattened, more or less round shape and a diameter of about 200 µm, but it appears elongated because of the laterally-connected, approximately 400 µm long perithecial neck, especially evident in longitudinal sections (fig. 3).

The perithecia contain long asci which measure 50-60 X 4-6 µm and whose eight ascospores are orientated towards the opening of the laterally-fixed ostiole. Mature ascospores are needle-shaped, have one slightly eccentric septum and measure 40-50 µm (fig. 4). The bundled spores are joined to the apical ring of the ascus by 10 µm long threads which can be longer after dissection.

Nomenclature

The first description of the fungus causing leaf blotch of maple was given in 1930 by von Tubeuf (8). Because the symptoms seemed similar to leaf blotch of plane-tree (caused by *Apiognomonia veneta* [Sacc. & Speg.] Höhn.) and based on the form of the perithecia and asci, von Tubeuf placed the fungus in the family Gnomoniaceae and gave it the name *Gnomonia pseudoplatani*. The single perithecia, immersed in substrate and protruding through the leaf surface with a black beak, were seen by him as a common feature of all Gnomoniaceae, but he also called for further investigation into systematics.

The extensive reorganization of the Diaporthales by Barr (1) in 1978 also included a taxonomical review of the Gnomoniaceae. Based on this work, Barrett and Pearce (2) in 1981 placed the leaf blotch fungus in the genus *Ophiognomonia* (Sacc.) Sacc., as *Ophiognomonia pseudoplatani* (v. Tubeuf) Barret & Pearce, not without remarking on the unusual placement of the perithecial neck. This laterally-connected ostiole and the more or less horizontal position of perithecia and asci in the leaf tissue, together with the spore-shape, seem to have caused Monod (7) to place the fungus in the genus *Pleuroceras* when he reviewed the Gnomoniaceae in 1981. Subsequently, the present name is *Pleuroceras pseudoplatani* (v. Tubeuf) Monod.

Anamorph

Of the few reports on leaf blotch of maple, none mentions reproductive fungal structures other than the perfect form; they even stress that imperfect forms cannot be found on leaves during the growing season (2, 7). Butin and Wulf (5) recently found fruiting structures similar to acervuli within the blotches, which were probably not recognized before because of their small size and inconspicuous appearance, and which can definitely be linked to the developmental cycle of *P. pseudoplatani*.

These fruiting bodies can be found from August onward on the blackened veins of the leaf undersides and also on the upper part of the petiole adjoining the leaf surface. Not visible to the naked eye, they appear as 100-350 X 50-200 µm small black blisters (fig. 2). Their pseudoparenchymal basal tissue develops interepidermally, the top layer being composed of bottle-shaped conidiogenous cells which produce club-shaped, straight or slightly curved, 6-7.5 X 2-3 µm phialoconidia. Because these conidia neither germiante on artificial substrate nor cause new infections following their production in late summer, they can be considered to be spermatia. Their function in the developmental cycle of the fungus could therefore be to dikaryotize the haploid mycelium prior to karyogamy and development of the perfect form. For the pathologist, they facilitate the identification of this fungal disease during the growing season.

Placing the newly found fungal form in the genus *Asteroma* DC. ex St. Ammans, as *Asteroma pseudoplatani* Butin & Wulf, is justified by conidiomata and fruiting-form, together with the fact that *Asteroma* is

assigned as the anamorph to *Pleuroceras* in scientific literature. Therefore, the discovery of this anamorph supports the latest taxonomical placement of the perfect form in the genus *Pleuroceras*.

Pure Culture

Although in earlier studies it was impossible to culture the fungus on various nutrient media (2), pure cultures are now successful. Both mycelium from moistly stored leaves and from ascospores yielded identical pure cultures, with a temperature optimum on different media of between 15 and 20 C. On malt agar, these cultures develop a flat, cottony aerial mycelium which appears sometimes with a pink hue, accompanied by dark gray spots and a hyaline to beige-colored and sectored edge. The hyphae are hyaline to olive-colored, smooth, 1-4 µm wide and contain granular inclusions.

Importance and Protective Measures

As with many other leaf diseases of deciduous trees, leaf blotch of maple poses no real threat to the affected tree. This disorder seems to become apparent only when weather conditions, especially during spring, strongly favor epidemic development. Besides a certain reduction in increment because of an up to 90 % coverage of leaf area, the leaf blotches cause aesthethic damage to urban trees.

Chemical protection certainly is not justified by this degree of damage and could be considered only by a high incidence in nurseries. On the other hand, there is the possibility of interrupting the disease cycle by removing leaves in autumn, as with many other leaf diseases. It was very obvious to the author that trees with considerable undergrowth were more heavily affected than "well cared for" trees along roads. Areas continuously attacked by leaf blotch should therefore consider the ecologically safe practice of removing leaves in autumn.

Acknowledgements

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Literature Cited

1. Barr, M.E., 1978: The Diaporthales in North America. Mycol. Memoir No. 7. Verlag J. Cramer, Lehre, 232 p.
2. Barret, D.K., Pearce, R.B. 1981. Giant leaf blotch disease of sycamore (*Acer pseudoplatanus*) in Britain. Trans. Brit. Mycol. Soc. 76:317-320.
3. Buchwald, N.F. 1957. Bildrag til Bornholms Svampeflora. Bornholms naturhistoriske Forenings jubilaeumsskrift 1957.
4. Butin, H., 1989. Krankheiten der Wald- und Parkbäume. Thieme Verlag, Stuttgart-New York, 216 p.
5. Butin, H., Wulf, A. 1987. *Asteroma pseudoplatani* sp.nov., Anamorphe zu *Pleuroceras pseudoplatani* (v. Tubeuf) Monod. Sydowia 40:38-41.
6. Laubert, R. 1927. Die Schwarzfleckenkrankheit des Ahorns. Biol. Reichsanst. f. Land-u. Forstw, Flugblatt Nr. 29.
7. Monod, M. 1983. Monographie taxonomique des Gnomoniaceae. Sydowia (Ann. Mycologici, Ser. II), IX. 315 p.
8. von Tubeuf, L. 1930. *Gnomonia pseudoplatani* n.sp., die Ursache der Riesenflecken auf den Blättern des Bergahorns (*Acer pseudoplatanus*). Z. Pflanzenkrankh. Pflanzensch. 40:364-375
9. Wulf, A. 1988. *Pleuroceras pseudoplatani* (v. Tubeuf) Monod, Erreger einer Blattbräune an Bergahorn (*Acer pseudoplatanus* L.). Nachrichtenbl. Deut. Pflanzenschutzd. 40(5):65-70.

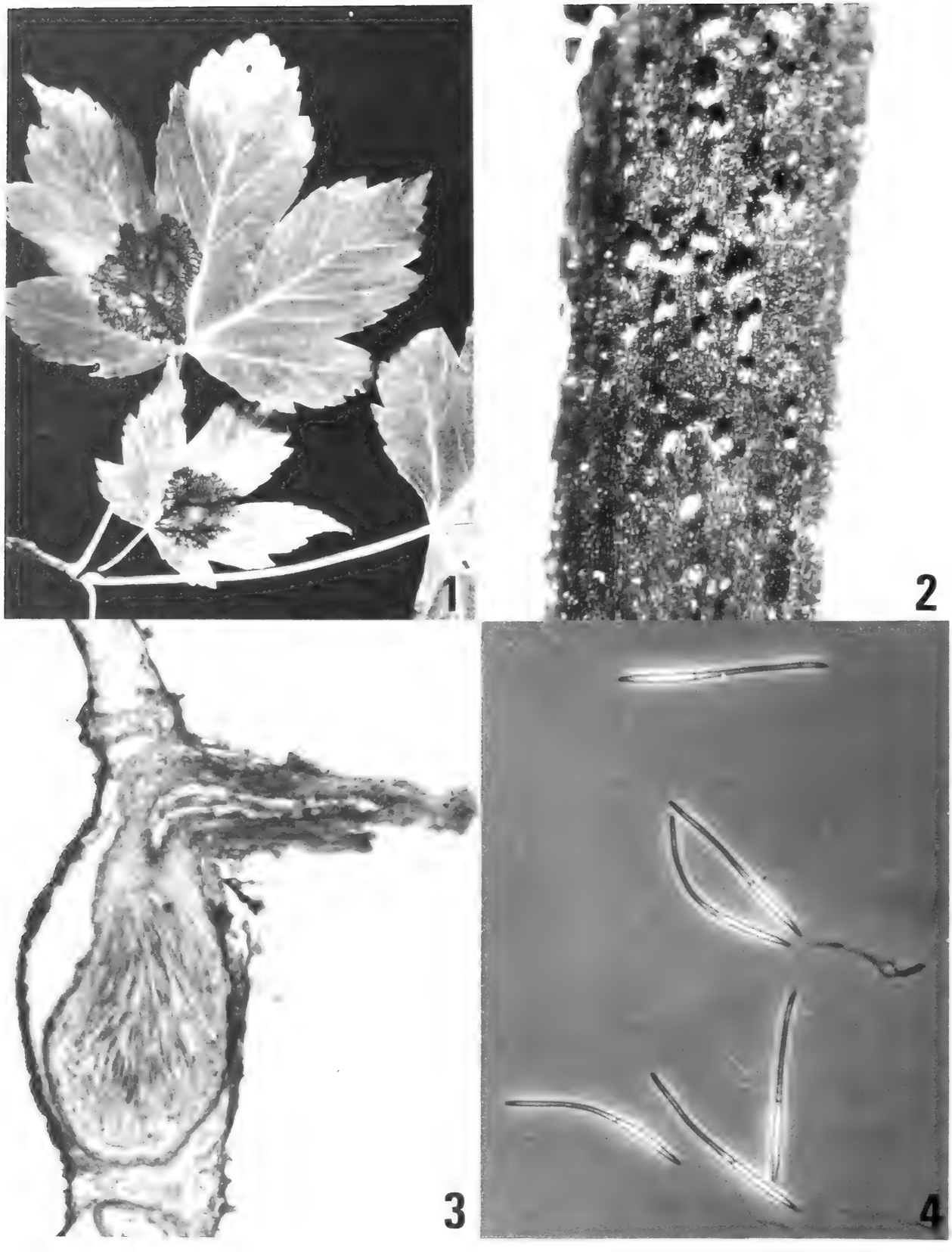

Figure 1.—Typical leaf blotches on maple.
Figure 2.—Acervuli of *Asteroma pseudoplatani*.
Figure 3.—Section through a perithecium of *Pleuroceras pseudoplatani*.
Figure 4.—Ascospores of *Pleuroceras pseudoplatani*.

Melampsora Leaf Rust on *Populus* in the North Central United States[1]

Robert W. Stack[2] and Michael. E. Ostry[3]

Abstract.—*Populus* species and hybrids are extensively used in fiber production plantations and for wind erosion protection and microlimate modification. Leaf rust, caused by *Melampsora* species, can reduce growth, impair hardiness or predispose poplars to other diseases or pests. In the northern United States several species of *Melampsora* infect poplars but little is known of their local distribution, their season-to-season occurrence, or their specificity on hybrid poplars. From 1980 through 1983 we made 169 leaf rust collections in five North Central States, Michigan, Minnesota, Iowa, North Dakota and Wisconsin. Of these rust collections, 158 were *M. medusae*, and eleven were either *M. abietis-canadensis* or *M. albertensis*. Over half of the non-*M. medusae* collections were from hybrid poplars. Hybrid poplar clones to be used in the northern U.S. should be tested for reaction to *Melampsora* species other than *M. medusae*.

Introduction

Trees in the genus *Populus* are widespread throughout North America; about 15 species are recognized. The trembling aspen, *P. tremuloides* Michx., has the most extensive distribution of any tree in North America. Other widely distributed species include cottonwood (*P. deltoides* Bartr. ex Marsh.), black cottonwood (*P. trichocarpa* Torr. & Gray), balsam poplar (*P. balsamifera* L.), and bigtooth aspen (*P. grandidentata* Michx.) (6). In addition to native species, the European white poplar (*P. alba* L.) is widely naturalized.

Populus species hybridize easily. This, coupled with their relative ease of vegetative propagation, has led to planting of interspecific hybrid clones for commercial forestry, wind and erosion control and ornamental use. Hybrid poplars capable of extremely rapid growth have been developed for management under extensive silvicultural systems in "maximum fiber production" plantations (5,6,31). Other hybrids have been selected to survive the adverse climates and alkaline soils of naturally treeless, windswept steppes, such as the North American Great Plains (32). For hybrid poplars to succeed in these uses they must be able to resist serious pests and diseases (16,23,24).

A major disease affecting native species and hybrids of *Populus* is leaf rust caused by *Melampsora* spp. This rust can cause premature defoliation, reducing biomass accumulation and vigor of affected trees. Defoliation may also delay or prevent proper hardening of terminal buds and shoots, predisposing them to winter injury. Severe defoliation for several successive years stresses trees, predisposing them to other damaging agents and may result in tree mortality (12,20,24,36).

Hybrid poplars intended for use in the north-central States and the northern Great Plains of the U.S. are evaluated for susceptibility to *M. medusae* Thüm., the prevalent rust in this region (4,15,16,23). However, some hybrid poplars resistant to *M. medusae* may be quite susceptible to one or more of the other *Melampsora* species. The rust species themselves may occasionally hybridize, introducing new virulences into the predominant species and infecting previously resistant poplar clones. The recent advent of biotechnological methods for disease resistance screening may inadvertently increase the risk of epidemics of "new" rust races by increasing chances of clones having exclusivelly vertical resistance. One of us (RWS) has recently discussed this possiblity in detail (28).

Poplar leaf rust is indigenous in North America. Arthur (1) listed five *Melampsora* species on *Populus*, each occurring on particular native or naturalized species. These *Melampsora* species were distinguished by their morphology, geographic distribution and host preference. There also are seven Eurasian species of *Melampsora* on poplars (9), but none of these has been reported in North America.

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Melampsora medusae is the most widely distributed species in North America, infecting trembling aspen and cottonwood (25). It has been reported in the United States and Canada from the Atlantic Coast westward to the Rocky Mountains and from northern Quebec and Ontario to the Gulf of Mexico (1,13,35). Recently, Shain (26) reported that *M. medusae* shows host specialization and proposed the names "f. sp. *deltoideae*" and "f. sp. *tremuloideae*" for the cottonwood and aspen forms, respectively. Clone-specific races of *M. medusae* have been reported in Australia and New Zealand and the phenomenon has been studied extensively (8,17,18,27).

The four other North American *Melampsora* species are of more limited occurrence. *Melampsora abietis-canadensis* (Farl.)Ludw. and *M. albertensis* Arth. occur in the northern United States and in Canada, *M. occidentalis* Jacks. occurs in western North America from the Rocky Mountains to the Pacific, and *M. aecidioides* (DC.)Schroet. is reported only along the eastern and western coasts where mild climates prevail (1,3,7,34,39).

On some of the native poplars, the four indigenous North American species of *Melampsora* show specificity or at least preference under natural condition. *Populus balsamifera* and *P. trichocarpa* are host to all four rusts, *P. deltoides* to *M. medusae* and *M. abietis-canadensis*, *P. grandidentata* to *M. albertensis* and *M. abietis-canadensis*, and *P. tremuloides* to *M. medusae*, *M. albertensis* and *M. abietis-canadensis* (1,3,7,34,39). Among *Populus* species, *M. aecidioides* is limited to the widely naturalized *P. alba*.

The present study was done to determine how frequently *Melampsora* species other than *M. medusae* occur in the north-central United States and to identify hybrid clones on which they occur. A brief preliminary report has been published (29).

Methods

Field collections.—All of the work reported here was done using field-collected, naturally infected leaves. Between 1980 and 1983, we made 169 separate collections of rusted leaves of *Populus* species and hybrids. Each collection consisted of several infected leaves from a single clone or single individual wild tree. There were twelve collections from Iowa, four from Michigan, 57 from Minnesota, 69 from North Dakota, and 27 from Wisconsin. Leaves were sent to Fargo for processing using a leaf mailer developed for this purpose (30). Leaves were dried and preserved as herbarium specimens. Complete sets of these collections will eventually be deposited at the J. C. Arthur Herbarium at Purdue University. For comparisons, voucher specimens of the five North American *Melampsora* species on *Populus* were obtained from the Arthur Herbarium and from the Canadian Forestry Service at Victoria, B.C.

Microscopy.—Portions of leaves from collections were prepared for scanning electron microscopy (SEM). On each dry leaf portion, four uredial pustules were randomly selected, mounted on stubs and examined by SEM. SEM micrographs were made of two different parts of each pustule, for a total of eight per sample. Prints at 1000X were prepared from each micrograph. Each print showed approximately 10 spores in sufficient detail to determine which appeared entirely echinulate and which had a smooth region (bald spot). The circumferential width of the bald spot, if present, was measured directly from the SEM prints; size of bald spot was also inferred from frequency of observation of spores showing or not showing it.

Size measurements were determined directly from the SEM pictures. Because dry uredospores (as imaged by SEM) are smaller than hydrated ones (as in wet mounts for light microscopy), the sizes taken from SEM must be adjusted (22). To determine the correction, spores from nineteen collections representing the entire range of spore sizes were mounted in water and size determined by light microscopy (LM). The SEM sizes were then compared to LM sizes to determine a correction factor. Collections determined to be species other than M. medusae were re-examined by LM to confirm measurements by SEM.

Results

Symptoms and signs on *Populus* leaves were typical of those described in the literature for *Melampsora* leaf rust (20,25,27). There was variation in infection type, lesion size and frequency, and sporulation, but none of these characters was adequate to distinguish infections caused by different *Melampsora* species.

When we compared uredospore size as measured by light microscopy and by SEM, the SEM spore sizes were consistently smaller, but the ratio between size of wet mounted spores measured by light microscopy and that of dry spores by SEM was relatively constant over the entire range (18 to 44 μm) of spore sizes. We found that length of dry spores determined by SEM was 61% ± 5% of their lengths measured by light microscopy. Sizes presented elsewhere in this paper are for hydrated spores, since this is the way measurements are presented in the other literature.

Uredospores of four *Melampsora* species are shown at the same magnification in figure 1. Differences in size between the very large *M. occidentalis*,

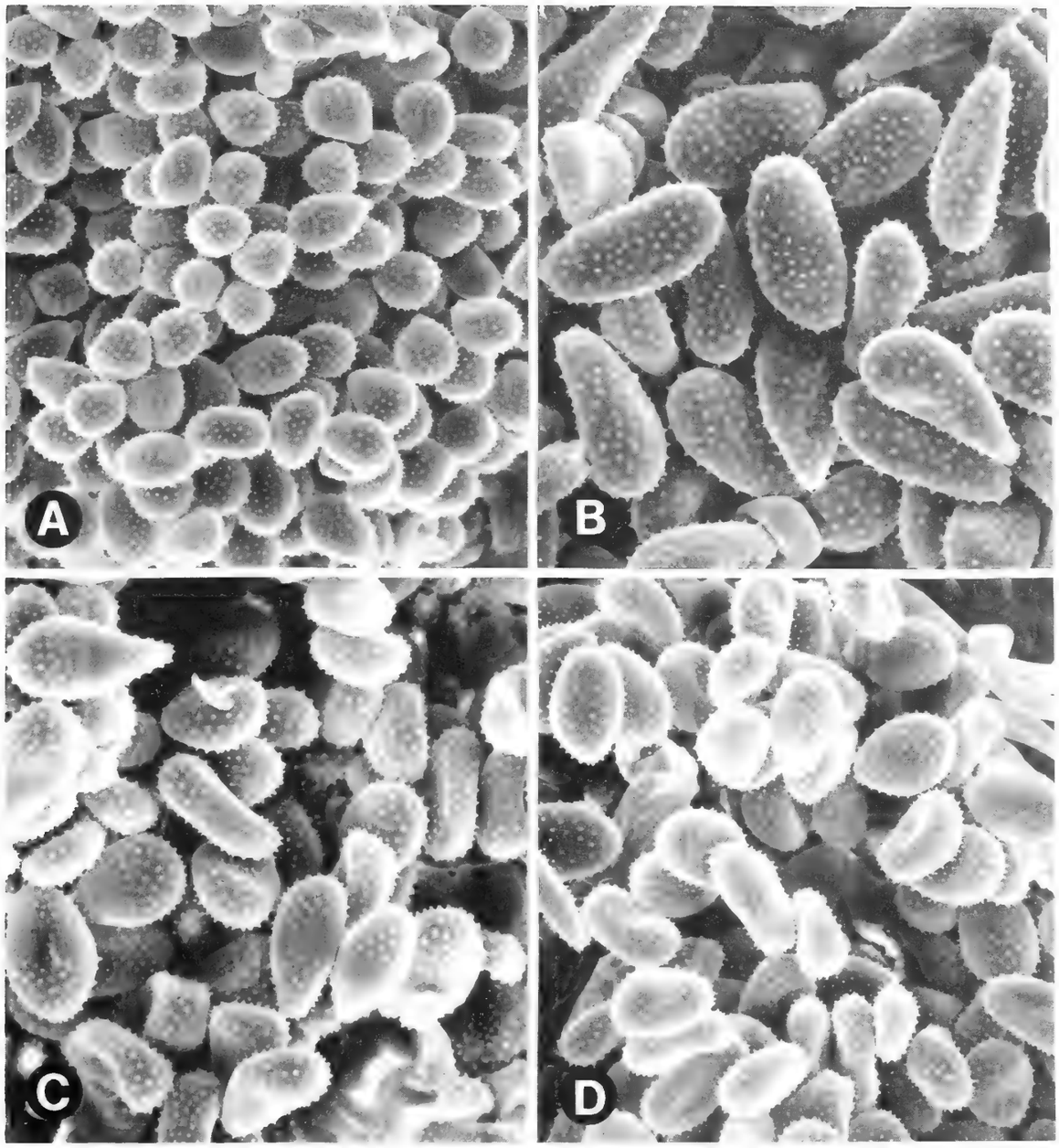


Figure 1.—Uredospores on uredia of four North American *Melampsora* species. A. *M. abietis-canadensis*. B. *M. occidentalis*. C. *M. medusae*. D. *M. albertensis*. (SEM X 1000)

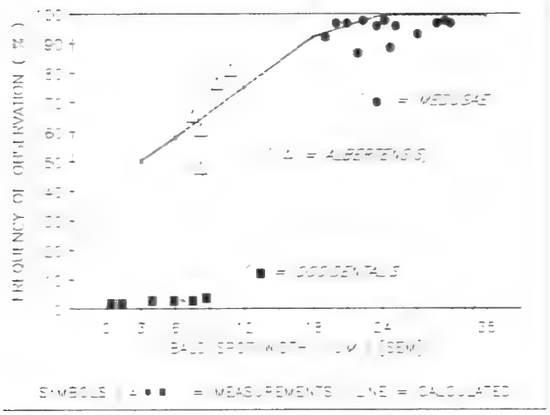
intermediate *M. medusae* and *M. albertensis* and the small *M. abietis-canadensis* are evident. Echinulation was very prominent in SEM photos. Spores which were spiny over their entire surface were easily distinguished from those having a bald spot. The uredospores of *M. medusae* (fig. 1C) show the prominent equatorial bald spot characteristic of this species while those of *M. occidentalis* (fig. 1B) and *M. abietis-canadensis* (fig. 1A) are entirely spiny, lacking a bald region.

Melampsora medusae and *M. albertensis* differ in the uredial stage mainly by the extent of the bald spot. This difference is visible by comparing figure 1C (*M. medusae*) and 1D (*M. albertensis*).

Since SEMs show only one side of any particular spore, the observation of a small feature present only at one point on the circumference becomes a matter of probability. The chances of a spot being seen in a sample of spores can be calcualted by spots of different sizes, and increase as the spots become larger. This calculated relationship is shown by the dotted line in figure 2. To see if this theoretical relationship would hold up, bald spots were measured from SEM photos.

The measured size and frequency of observation for each sample were then plotted against the theoretical line. Reasonably good agreement was found (fig. 2).

FIGURE 2.
RELATION OF SIZE OF BALD SPOT AND FREQUENCY OF VISIBILITY IN SEM PHOTOS OF UREDOSPORES

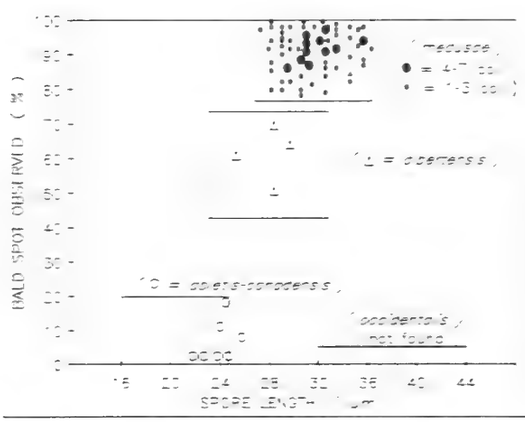


Any spot should be visible roughly half the time assuming the spores are randomly positioned. This sets the lower limit of observation frequency for spores on which a bald spot is regularly present. Samples on which only an occasional bald spot occurred would show lower frequencies or indicate mixtures.

Use of measurements for uredospores alone can give a near-unique separation to the *Melampsora* species when they are represented as ordinates. Based on uredospore measurements, the identity of the 169 poplar rust collections examined is shown in figure 3. Among the 169 collections, 158 were identified as *M. medusae*, seven as *M. abietis-canadensis*, and four as *M. albertensis*. Identity of *M. albertensis* was confirmed by examining sections of teliospores. *Melampsora occidentalis* and *M. aecidioides* were not found in these collections.

Of the four collections identified as *M. albertensis*, one was found in 1981 from Wisconsin and three in 1982, one each from Minnesota, North Dakota and Wisconsin. Of the seven collections identified as *M. abietis-canadensis*, two were found in 1981 in Wisconsin, and five were found in 1982, three from Wisconsin and one each from Michigan and North Dakota. *Melampsora abietis-canadensis* was not found in Minnesota collections (57) in either year. Neither species was found in collections (12) from Iowa.

FIGURE 3.
MELAMPSORA COLLECTIONS 1980 - 1983 FROM THE NORTH CENTRAL STATES



Of collections of *M. abietis-canadensis*, three were on native *Populus* spp., once each from bigtooth aspen (*P. grandidentata*), balsam poplar (*P. balsamifera*), and trembling aspen (*P. tremuloides*) and once from an exotic species, *P. tremula* L. all in Wisconsin. Both *M. albertensis* and *M. abietis-canadensis* were collected from hybrid poplar clones.

Two leaves examined contained pustules of both *M. medusae* and *M. albertensis* and two others had both *M. medusae* and *M. abietis-canadensis*. All four mixed infections were from hybrid poplars.

Discussion

Our finding of mixed infections on single leaves in several instances suggests that mass field collections of spores for controlled inoculations may well contain mixtures, even if such collections are restricted to single plants or clones. Mixed infection on hybrid poplars has been reported previously (14).

Ziller (37,38) extentensively studied both the aecial and telial host ranges of *Melampsora* and concluded (39) that *M. albertensis* should by synonymous with *M. medusae*. We believe, based on present work, that *M. albertensis* can be distinguished from *M. medusae* with resonable certainty from uredospore collections. Admittedly the numbers of *M. albertensis* included here are low, and more extensive collections might reveal a continuum of bald spot sizes. The retention of *M. albertensis* rests, however, not just on this one character (bald spot size) but on another, more defined one, the thickness of the upper wall of the teliospore, which allows a complete separation of the two species (1).

Clarification of the distribution of and host susceptibility to these several rusts will facilitate study of pathogenic variation in *M. medusae* and help avoid use of hybrid poplars tested for resistance to that species but highly susceptible to the other rust species in areas where these are likely to occur. With further study it might be possible to identify "indicator" clones which are highly susceptible to individual *Melampsora* species similar to the procedure widely used to detect pathogenic races of rusts in agronomic crops. These indicators could be included in nurseries and provenance plantings to monitor rust species occurrence. This type of procedure is routinely used

in wheat nurseries where highly rust-susceptible cultivars are always included to monitor the ambient inoculum levels.

Occurrence of pathogenic races of *M. medusae* has been reported from Australia and New Zealand (2,8,17,18,19). In the Southern hemisphere both the rust and *Populus* hosts are introduced so there are no natural epidemics on wild native hosts as occurs in North America (25,35). Epidemics on nonselected wild hosts tend to stabilize pathogen population. Several papers at a recent symposium discussed this aspect of host-parasite population genetics at length (10). Pathogenic races of *M. medusae* are not necessarily predominant in North America. One recent report concluded that races are probably absent in *M. occidentalis*, another indigenous rust on a wild *Populus trichocarpa* population (11). Extensive discussion of host-parasite interactions in unselected plants is given by Robinson (21). While pathogenic races of *M. medusae* may well occur in North America, their detection (and management) is complicated by the presence of several other indigenous rust species on a *Populus* (1) with overlapping geographic and host ranges and by the possible presence of formae speciales or host specific biotypes (26).

It is important to be able to separate the *Melampsora* species based on collections of uredospores alone because collections of spores preserved for use in inoculations will contain only these. We have shown that the non-*M. medusae* rusts occur at low levels (here about 6.5%) in the overall population of rusts in the northern U.S. and that more than one species may be present at once on a single tree or even a single leaf. Occasional, casual collections are likely to miss species other than the dominant one, leading to a false assumption that only one species is present. Bulk spore collections, even when from single trees or leaves, may contain admixtures of nondominant rust species; using such mixed spores to test *Populus* clones could lead to erroneous or confusing results.

Acknowledgements

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Literature Cited

1. Arthur, J. C., Cummins, G. B. 1962. Manual of the Rusts in United States and Canada. Hafner Publ. New York. 462 p.
2. Chandrashekar, M., Heather, W. A. 1980. Reaction of poplar clones to physiologic races of *Melampsora larici-populina* Kleb. Euphytica 29:401-407.
3. Conners, T. L. 1967. An annotated index of plant diseases in Canada. Can. Dept. Agric. Res. Branch Publ. 1251. 381 p.
4. Cooper, D. T., Filer, T. H. 1977. Geographic variation in *Melampsora* rust resistance in eastern cottonwood in the lower Mississippi Valley. Proc. Central States Forest. Tree Improv. Conf. 10:146-151.
5. DeByle, N. V., Winokur, R. P. (eds.) 1985. Aspen: Ecology and Management in the Western United States. USDA For. Serv. Gen. Tech. Rep. RM-119. 283 p.
6. Fowells, H. A. 1965. Silvics of Forest Trees of the United States. USDA Agric. Handb. 271. 762 p.
7. Ginns, J. H. 1986. Compendium of plant disease and decay fungi in Canada 1960-1980. Agric. Canada Research Branch Publ. 1813. 416 p.
8. Heather, W. A., Chandrashekar, M., Sharma, J. K. 1980. Forms and degrees of resistance in *Populus* spp. to the *Melampsora* leaf rusts occurring in Australia. Aust. For. 43:52-57.
9. Hennebert, G. L. 1964. L'Identification des rouilles du peuplier. Agricultura (Louvain) 12:661-670.
10. Heybroek, H. M., Stephan, B. R., Weissenberg, K. von 1982. Resistance to diseases and pests in forest trees. PUDOC, Wageningen, Netherlands. 501 p.
11. Hsiang, T., Kamp, B. J.van der. 1985. Variation in rust virulence and host resistance of *Melampsora* on black cottonwood. Can. J. Plant Path. 7:247-252.
12. Hubbes, M., Jeng, R. S., Zsuffa., L. 1983. Melampsora rust in poplar plantations across southern Ontario. Plant Dis. 67:217-218.
13. Jokela, J. J. 1966. Incidence and heritability of *Melampsora* rust in *Populus deltoides* Bartr. p. 111-117. In H. D. Gerhold et al. (ed.), Breeding Pest-resistant Trees. Pergamon Press, London.

14. Kraayenoord, C. W. S. van, Wilkinson, A. G. 1976. The Role of *P. deltoides* in New Zealand. p. 176-188. In Proc. Symp. on Eastern Cottonwood and Related Species. B A. Theilges and S. G. Land, Jr. (eds.) Louisiana St. Univ., Div. Cont. Educ., Baton Rouge.

15. Ostry, M. E., McNabb, H. S. Jr. 1985. Susceptibility of *Populus* species and hybrids to disease in the North Central United States. Plant Dis. 69:755-757.

16. Ostry, M. E., McNabb, H. S., Jr. 1986. Populus species and hybrid clones resistant to *Melampsora, Marssonina* and *Septoria*. USDA For. Serv. Res. Pap. NC-272. 7 p.

17. Prakash, C. S., Heather, W. A. 1986. Inheritance of resistance to races of *Melampsora medusae* in *Populus deltoides*. Silvae Genetica 35:74-77.

18. Prakash, C. S., Heather, W. A. 1986. Relationship between increased virulence and the aggressiveness traits of *Melampsora medusae*. Phytopathology 76:266-269.

19. Prakash, C. S., Thielges, B. A. 1987. Pathogenic variation in *Melampsora medusae* leaf rust of poplars. Euphytica 36:563-570.

20. Peterson, G. W., Stack, R. W. 1986. Leaf rust of poplar and willow. p. 4-5. In: Diseases of Trees in the Great Plains. USDA For. Serv. Gen. Tech. Rep. RM-129.

21. Robinson, R. A. 1976. Plant Pathosystems. Spring-Verlag. Berlin. 184 p.

22. Schimming, W. K., Littlefield, L. J. 1985. Size and shape of urediniospores under natural atmospheric conditions. (Abstr.) Phytopathology 75:1314.

23. Schipper, A. L., Jr. 1976. Foliage diseases of periodic importance to *Populus deltoides* and its hybrids. p. 234-244. In: B. A. Thielges and S. G. Land, Jr. (eds.) Proc. Symp. on Eastern Cottonwood and Related Species. Louisiana St. Univ., Div. Cont. Educ., Baton Rouge. 485 p.

24. Schipper, A. L., Dawson, D. H. 1974. Poplar leaf rust—a problem in maximum wood fiber production. Plant Dis. Rep. 58:721-723.

25. Shain, L. 1976. Etiology, epidemiology and control of *Melampsora* rust of cottonwood. p. 189-198. In: B. A. Thielges and S. B. Land, Jr. (eds.) Poc. Symp. on Eastern Cottonwood and Related Species. Louisiana St. Univ., Div. Cont. Educ., Baton Rouge. 485 p.

26. Shain, L. 1988. Evidence for formae speciales in the poplar leaf rust fungus, *Melampsora medusae*. Mycologia 80:729-732.

27. Sharma, J. K., Heather, W. A. 1979. Comparison of disease parameters for quantitative assessment of *Melampsora* leaf rust in clones of Populus species. Trans. Brit. Mycol. Soc. 72:483-488.

28. Stack, R. W. 1987. Long generation times in breeding trees, a pest management blessing in disguise. Proc. North Central Tree Impr. Conf. 5:72-81.

29. Stack, R. W., Ostry, M. E. 1983. Leaf rust on hybrid poplars in the North Central Region. (Abstr.) Phytopathology 73:837.

30. Stack, R. W., Ostry, M. E., Littlefield, L. J. 1984. A convenient holder for mailing and storing collected leaf specimens. USDA For. Serv. Res. Note NC-319. 2 p.

31. Thielges, B. A., Land, S. B. Jr. (eds.) 1976. Proc. Symp. on Eastern Cottonwood and Related Species. Louisiana State Univ., Div. Cont. Educ., Baton Rouge. 485 p.

32. Tinus, R. W. (ed.) 1976. Shelterbelts on the Great Plains. Great Plains Ag. Council Publ. 78. 218 p.

33. Toole, E. R. 1967. *Melampsora medusae* causes cottonwood rust in lower Mississippi Valley. Phytopathology 57:1361-1362.

34. USDA. 1960. Index of Plant Diseases in the United States. U.S. Dept. Agric. Agric. Handb. 165. 531 p.

35. Widin, K. D., Schipper, A. L. Jr. 1980. Epidemiology of *Melampsora medusae* leaf rust of poplars in the north central United States. Can. J. For. Res. 10:257-263.

36. Widin, K. D., Schipper, A. L. Jr. 1981. Effect of *Melampsora medusae* leaf rust infection on yield of hybrid poplars in the north-central United States. Eur. J. For. Path. 11:438-448.

37. Ziller, W. G. 1955. Studies of western tree rusts II. *Melampsora occidentalis* and *M. albertensis*, two needle rusts of Douglas fir. Can. J. Bot. 33:177-188.

38. Ziller, W. G. 1965. Studies of western tree rusts. VI. The aecial host ranges of *Melampsora albertensis, M. medusae*, and *M. occidentalis*. Can. J. Bot. 43:217-230.

39. Ziller, W. G. 1974. Tree Rusts of Western Canada. Can. Dept. Environment, For. Serv., Ottawa. 272 p.

Incidence of Melampsora Rust in a Seedling Plantation of Hybrid Poplar[1,2]

Tom Hsiang and Gary A. Chastagner[3]

Abstract.—Over 4800 hybrid poplar seedlings were surveyed for incidence of Melampsora rust in fall of 1988. Height and diameter measurements were also made on these 1-yr-old seedlings. The taxonomic lines involved were *Populus trichocarpa, P. maximowiczii,* and *P. deltoides*. There was a great deal of variation in growth and rust resistance between the different hybrid poplar families. Only seedlings with *P. trichocarpa* ancestry showed any susceptibility to *Melampsora occidentalis*. The twelve crosses of *P. maximowiczii* x *P. maximowiczii* showed no rust at all, and grew over double the volume of pure *P. trichocarpa* families. In three-way hybrid crosses between *P. maximowiczii* and hybrids of *P. trichocarpa* x *P. deltoides* (6 crosses), rust incidence was very low, with less than 1% of the seedlings showing a few rust spots on some leaves. Average growth of these three-way hybrid families was three times greater than the average growth of the pure *P. trichocarpa* families. In crosses between *P. trichocarpa* and *P. maximowiczii* (20 crosses), the majority of the seedlings had no rust, but roughly 25% had a few specks of rust, and 7% were noticeably more infected. Average growth of these hybrid families was greater than that of any other taxonomic grouping. The parental *P. trichocarpa* clones originating from east of the Cascade Mountains in Washington State conferred greater rust susceptibility to their progeny, with a 10% greater proportion of severely infected seedlings than progeny of westside clones. In addition to more rust, the pure eastside progeny, when grown west of the Cascade Mountains, had much less growth than pure westside progeny. Based on host range and spore size, the Melampsora rust found in this plantation was considered to be *M. occidentalis*.

Introduction

The genus *Populus* contains between 30 and 40 species belonging to several taxonomic sections. Poplar species are naturally found throughout the temperate and subtropical regions of Asia, Europe, North Africa and North America (6). Poplars are some of the most desirable tree species for intensive biomass production. Their rapid growth, easy clonal propagation, and quick resprouting after harvest make them ideal species for short rotation intensive culture (SRIC). SRIC refers to wood production in carefully tended plantations on productive sites for rotations of 3-10 years using fast growing hardwoods of good coppicing ability (5).

Ranney et al. (7) reviewed SRIC in the United States. They remarked upon the spectacular increase in productivity of one hybrid poplar breeding project (3) achieving yields of 27.6 Mg/ha/yr compared to other hybrid poplars (20.3 Mg/ha/yr) and native black cottonwood (12.5 Mg/ha/yr). This poplar breeding project is part of a larger productivity program which has been continuing for over 10 years and involves scientists at Washington State University and the University of Washington. Initially, studies in this project were carried out on the silviculture and breeding of poplars, but in recent years other areas, including physiology and molecular biology, have been added. Pathology is the most recent addition.

The use of hybrid poplars for fiber is increasing in the Pacific Northwest. Historically, most of the fiber in the Pacific Northwest has been supplied as a byproduct of the solid wood products industry. The availability of this fiber has been directly dependent on the demand for wood products. About 15 years ago, the pulp industry in the Pacific Northwest began using alder (*Alnus rubra* Bong.), which lent greater quality to certain paper products. Due to the recent high demand for alder and its past treatment as a weed species, a shortage of this material is foreseen. Pulp companies thus are considering hybrid poplars as a source of hardwood fiber (Brian Stanton, James River Corporation, personal communication).

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In 1981, a major forest product company in our area, Crown Zellerbach Corporation, now James River Corporation, initiated plot trials with the new cottonwood hybrids. They now have over 4000 ha of poplar plantations in the lower Columbia River region, and are developing 4000 ha in the mid-Columbia basin of Washington State. They also have other test plantings in Oregon (Brian Stanton, James River Corporation, personal communication). Other companies on the west coast of North America investigating the use of hybrid poplars include Weyerhauser Corporation, Scott Paper Ltd. and MacMillan Bloedel Ltd. (10).

The major purpose of this study was to examine seedlings of hybrid poplar for incidence and severity of Melampsora rust, caused by *Melampsora occidentalis* Jacks. The taxonomic lines involved were *Populus deltoides* Bartr. (eastern cottonwood), *P. trichocarpa* Torr. & Gray (black cottonwood) and *P. maximowiczii* Henry. *Populus deltoides* has a large range, covering the eastern half of the United States. It occurs extensively in pure and mixed stands, and many of its cultivars are in commercial use. *Populus trichocarpa* is a native poplar and the fastest growing tree of the Pacific Northwest. It ranges from California to Alaska and inland to the Rocky Mountains. *Populus maximowiczii* is native to Japan, Korea and eastern Siberia. It is a fast-growing tree attaining large size in natural mixed forest stands (12).

Populus maximowiczii was included in the UW-WSU poplar breeding program for its better performance on lower fertility sites and in cold climates, as well as greater resistance to rodents and defoliators (10). Although it is known to be susceptible to *M. larici-populina* Kleb. (2), the susceptibility of *P. maximowiczii* to *M. occidentalis* had not been characterized.

Melampsora occidentalis is a native pathogen causing leaf rust on black cottonwood. *Melampsora medusae* Thuem. can also be found in the Pacific Northwest on trembling aspen, *P. tremuloides* Michx., but does not infect black cottonwood (13). Melampsora leaf rust has been shown to cause biomass losses of over 50% on black cottonwood cuttings after the first growth season (J. Wang & B. van der Kamp, University of British Columbia, unpublished data), and has been observed to cause mortality to first year cuttings of highly susceptible *P. trichocarpa* clones (P. Heilman, Washington State University, personal communication). However, very little research has been done on the *Melampsora occidentalis* - *Populus trichocarpa* pathosystem. Hsiang & van der Kamp (4) found tremendous differences in pathogen virulence and even more in host resistance of wild black cottonwood trees.

The purpose of this study was to survey hybrid poplar seedlings for the incidence and severity of rust caused by *M. occidentalis*, as part of a breeding and selection program, and to gain basic information on the rust resistance of *P. maximowiczii* and its hybrids in the Pacific Northwest. A secondary objective was to examine isolates of *Melampsora* from various *Populus* species and hybrids, and determine pathogen species locally present based on spore size and host range.

Materials and Methods

In 1987, the breeding program of the University of Washington-Washington State University poplar program emphasized two major aspects: hybridization of *P. trichocarpa* females with *P. maximowiczii* (20 crosses), and crosses among selected clones of *P. trichocarpa* from east and west of the Cascade Mountains (29 crosses). Other families included in this study were 12 crosses of *P. maximowiczii* by *P. maximowiczii*, three of (*P. trichocarpa* x *P. deltoides*) by *P. maximowiczii*, and three of *P. maximowiczii* by (*P. trichocarpa* x *P. deltoides*) (11).

Controlled pollination crosses were carried out in spring of 1987. *Populus maximowiczii* seeds and pollen were obtained from Dr. Chiba of the Oji Paper Company in Japan. When possible, over 300 seeds of each cross were planted in wooden flats and grown in an outdoor protected area beginning in summer, 1987. Up to 100 seedlings of each cross were planted in spring, 1988, at Washington State University Farm 5 near Puyallup. They were placed in a non-random row pattern between two older stands of hybrid poplar. Although this design did not permit statistical analysis of differences between clones, entire families could be compared, particularly since there were many parental clones in common in the different families.

In the fall of 1988, rust severity ratings and height and diameter measurements were made on approximately 4800 seedlings in the above planting. Melampsora rust ratings followed the system of Schreiner (8). The most heavily infected foliage was rated in classes of 0 (no rust), 1 (light), 5 (medium), or 25 (heavy), and the amount of foliage at this rating was noted as 1 (<25%), 2 (25% to 50%), 3 (50% to 75%), or 4 (>75%). The final disease rating was the product of these two measurements, giving a final scale of 0 (no infection) to 100 (severely infected). Statistical analyses were performed on the measurements using SAS-PC, and comparisons were made between *P. trichocarpa* families of east or westside origin, and between different hybrid taxons. Volume was calculated as height x diameter^2, not accounting for seedling form.

In addition to these field surveys, an attempt was made to determine the identity of the Melampsora rusts. In September, 1988, *Melampsora* isolates were collected from some hybrid poplar seedlings at the

WSU-Farm 5 site plus from two Farm 5 hybrids of *P. trichocarpa* x *P. deltoides*, an eastside *P. trichocarpa* and a trembling aspen from Orting, Washington. These isolates were inoculated onto leaves of several greenhouse-grown hybrid poplars, including a "universal suscept" to *M. medusae* obtained from Drs. C. Prakash & B. Thielges of the University of Kentucky, namely *P.* x *euramericana* cv. I-488 (a hybrid of *P. deltoides* and *P. nigra* L.). Spore sizes were measured for the aspen isolate and two *P. trichocarpa* isolates, and compared with published values.

Results and Discussion

All hybrid families.—Regarding the incidence of rust on the various hybrids, the most striking feature was the absence of rust on seedlings of *P. maximowiczii* (table 1). Hybrids of *P. maximowiczii* with *P. trichocarpa* as the female parent showed very low levels of rust, but infected progeny occurred in all these hybrid families. Roughly 25% of these hybrid seedlings had a few specks of rust, and 7% were noticeably more infected.

Hybrid vigor was most evident in growth of hybrids of *P. trichocarpa* x *P. maximowiczii* (table 1). With respect to volume, these interspecific hybrids grew 225% better than the pure *P. trichocarpa* seedlings and 42% better than pure *P. maximowiczii* seedlings. Many parents were shared in common between the different hybrid families.

The three-way hybrids, containing germplasm of *P. trichocarpa, P. deltoides*, and *P. maximowiczii*, were also highly resistant to rust. In three out of six of these three-way hybrid families, there was no rust on any of the seedlings; the overall rust incidence was less than 1%. Volume growth was also exceptional with these three-way hybrid families, surpassing those of *P. trichocarpa* or *P. maximowiczii* families.

In this study, no causation can be implied between rust and growth, but there was a significant correlation ($P < 0.05$) between rust severity and volume growth ($r^2 = 0.32$). When rust severity values were transformed as an inverse function (SEVERITYnew = 1/(SEVERITYold+1), the correlation increased to $r^2 = 0.44$. In any case, first generation *P. trichocarpa* x *P. maximowiczii* hybrids with greater rust resistance and growth may prove to be excellent material for SRIC in the Pacific Northwest.

Populus trichocarpa families. — When considering families of *P. trichocarpa* parentage only, there were strong differences between clones from east or west of the Cascade Mountains (table 1). The westside has a moist maritime environment, while the eastside has a dry continental climate. Hybrid families with any eastside germplasm had, on average, twice the rust severity rating as pure westside clones. Pure eastside progeny had a 10% greater proportion of seedlings in the heavily infected category than pure westwide progeny. This phenomenon was previously observed in British Columbia by Hsiang and van der Kamp (4) who found that eastside clones had greater susceptibility to both westside and eastside isolates of *M. occidentalis* than did most westside clones. A possible

Table 1.—Means of family height, diameter, volume and rust rating of hybrid poplar seedlings

Taxon[1]	Number of Families	Number of Seedlings	Height (cm)	Diameter (mm)	Volume[2] (cm^3)	Rust[3] Rating
All poplar famlies						
MxM	12	571	194 a[4]	14.8 b	594 c	0 b
TDxM	3	150	183 b	16.4 a	837 a	0 b
MxTD	3	207	171 c	14.9 b	701 b	0 b
TxM	20	1666	201 a	17.4 a	846 a	0.4 b
TxT	29	2080	138 d	11.4 c	260 d	7.0 a
P. trichocarpa only						
ExE	2	189	97 d	7.7 c	87 d	8.3 ab
ExW	7	440	128 c	10.6 b	189 c	9.5 a
WxE	14	1140	139 b	11.6 b	254 b	6.8 b
WxW	6	286	174 a	13.8 a	445 a	3.8 c

[1]The taxons of poplar are abbreviated as follows: T = *P. trichocarpa*, M = *P. maximowiczii*, TD = hybrids of T and *P. deltoides*, E = eastside *P. trichocarpa*, and W = westside *P. trichocarpa*.

[2]Volume = height x diameter x diameter, and does not account for taper and form of the seedling.

[3]Rust rating followed the method of Schreiner (8) where 0 = no rust and 100 = severely infected.

[4]Means in vertical columns of each section followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

explanation is that eastside clones are genetically more susceptible, since high levels of resistance may be less necessary on the eastside where conditions for rust cycling are unfavorable during the hot dry summers.

As for height, diameter and volume growth, correlations between these and transformed rust severity values were $r^2 = 0.48$, $r^2 = 0.50$, and $r^2 = 0.52$, respectively. Again no causation can be implied, but it is interesting to note that families with eastside germplasm had greater rust severity and also less growth than westside families.

Present and future experiments with these *P. trichocarpa* families include replicated field trials supplemented by in vitro inoculation tests. For the field trials, randomly selected progeny of several families have been planted and exposed to high levels of inoculum. These will be evaluated in late summer 1989 and again in 1990. Another replicated planting has been set up to study the etiology of Septoria leaf blight caused by *Mycosphaerella populorum* Thomp.

Identification of rust isolates.—An isolate from a hybrid poplar plantation east of the Cascade Mountains near Wenatchee, Washington, caused infections when inoculated onto leaves of black cottonwood. Farm 5 isolates from hybrid seedlings (*P. trichocarpa* with either *P. deltoides* or *P. maximowiczii*) proved to be pathogenic on leaves of pure *P. trichocarpa*, but not pathogenic on *P.* x *euramericana* cv. I-488. Based on pathogenicity and spore size (table 2), these isolates were considered to be *M. occidentalis*.

The *Melampsora* isolate obtained from an infected trembling aspen leaf had dimensions quite distinct from the black cottonwood isolates. Based on spore size, this isolate appeared to be *M. medusae* (table 2). When inoculated onto leaves from greenhouse-grown cuttings of various hybrid poplars, this aspen isolate caused no infections. Attempts to infect cv. I-488 with the aspen isolate also failed, although this may have been due to the fact that relatively young leaves were used, and young poplar leaves tend to be rust resistant (9). The aspen isolate could not be maintained through fall 1988, since attempts to root aspen cuttings failed. Future tests will attempt to establish whether the local aspen Melampsora rust is the same *M. medusae* that is found in the Midwest on eastern cottonwood and hybrid poplars there.

Table 2.—Sizes (means and standard errors) of rust spores obtained from the foliage of *Populus* species[1]

	Length	Width
Orting aspen (*M. medusae*)	29.5±1.0 µm	19.6±0.6 µm
Farm 5 hybrid (*M. occidentalis*)	43.6±1.7 µm	24.4±0.8 µm
Eastside hybrid (*M. occidentalis*)	48.1±1.0 µm	25.9±0.4 µm

[1]Arthur & Cummins (1) and Ziller (13) reported that *M. medusae* uredospores were 26-35 X 16-23 µm, and *M. occidentalis* 32-48 X 16-27 µm.

In summary, we observed a great deal of variation in rust resistance and amount of growth between different hybrid poplar families. Those families with *P. maximowiczii* germplasm had very high to total rust resistance against our local races of Melampsora rust. In addition to inferior growth, clones of pure *P. trichocarpa* lineage varied in rust resistance depending on where they or their ancestors originated. *Populus trichocarpa* clones from west of the Cascade Mountains had higher resistance to local rust races than those from the eastside. Based on spore size and pathogenicity, our local isolates of *Melampsora* from poplars are likely to be *M. occidentalis*.

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Literature Cited

1. Arthur, J. C., Cummins, G. B. 1962. Manual of the Rusts in United States and Canada. Hafner Publ. Co., New York, 438 p. + suppl.

2. Chiba, O. 1964. Nature of resistance of poplar clones to a leaf rust, *Melampsora larici-populina*. p. 207-220. In: H. D. Gerhold et al. (eds.), Breeding Pest Resistant Trees, Pergammon Press, Toronto, 505 p.

3. Heilman, P. E., Stettler, R. F. 1985. Genetic variation and productivity of Populus trichocarpa T. & G. and its hybrids. II. Biomass production in a 4-year plantation. Can. J. For. Res. 15:384-388.

4. Hsiang, T., van der Kamp, B. J. 1985. Variation in rust virulence and host resistance of *Melampsora* on black cottonwood. Can. J. Plant Path. 7:247-252.

5. Isebrands, J. G., Nelson, N. D., Dickmann, D. J., Michael, D. A. 1983. Yield physiology of short rotation intensively cultured poplars. USDA For. Serv. Gen. Tech. Rep. NC-91, p. 77-93.

6. Khosla, P. K., Khurana, K. D. 1982. Evolution of genus *Populus* Linn. and systematic placement of *P. ciliata* Wall. ex Royle. J. Tree Sci. 1:81-87.

7. Ranney, J. W., Wright, L. L., Layton, P. A. 1987. Hardwood energy crops: the technology of intensive culture. J. For. 85(9):17-28.

8. Schreiner, E. 1959. Rating poplars for Melampsora leaf rust infection. USDA For. Serv. Res. Note NE-90, 3 p.

9. Sharma, J. K., Heather, W. A., Winer, P. 1980. Effect of leaf maturity and shoot age of clones of Populus species on susceptibility to *Melampsora larici-populina*. Phytopathology 70:548-554.

10. Stettler, R. F., Hinckley, T. H., Heilman, P. E. 1988. Genetic improvement and evaluation of black cottonwood for short rotation biomass production. Ann. Tech. Rep., Univ. Washington-Washington State Univ. Poplar Program. 133 p. + App.

11. Stettler, R. F, Hinckley, T. H., Heilman, P. E. 1989. Genetic improvement and evaluation of black cottonwood for short rotation biomass production. Ann. Tech. Rep., Univ. Washington-Washington State Univ. Poplar Program. 129 p.

12. Thielges, B. A. 1986. Breeding poplars for disease resistance. FAO Forestry Paper 56. 66 p.

13. Ziller, W. G. 1974. The Tree Rusts of Western Canada. Can. For. Serv., Dept. Environ. Publ. 1329, 272 p.

Occurrence and Pathogenicity of *Gnomoniella Fraxini*, Cause of Ash Anthracnose[1]

Robert W. Stack, Teresa E. Snyder and Scott C. Redlin[2]

Abstract.—*Gnomoniella fraxini* was isolated from a range of symptomatic tissues in green ash leaves, fruits and stems. All cultures were similar in growth and pathogenicity. Survey of a green ash monoculture showed disease occurred in foci. Female trees showed more severe disease than male trees. Clinging of infected petioles into the following spring was prominent on trees with a history of anthracnose.

Introduction

Ash anthracnose is widespread throughout North America (4,20) and has been called the most common foliage disease of ash in the United States. Ash anthracnose is caused by *Gnomoniella fraxini* Redlin and Stack, anamorph: *Discula fraxinea* (Peck) R&S (= *Gloeosporium aridum* E&H)(15). Ash anthracnose is confined to species of *Fraxinus*. Green ash, *F. pennsylvanica* Marsh, has the widest natural distribution of any species in North America. Its native range extends from Nova Scotia on the Atlantic coast westward into Alberta in Canada and southward across the United States to central Texas and northern Florida (5,18). In the northern Great Plains, green ash is extensively used due to its tolerance of cold temperatures, high soil pH and drought, factors which seriously limit species selection for plantings of any kind in this region (7).

In general, anthracnose diseases of hardwood trees are more important in boulevard and landscape plantings (11,13) and plantations (1) and less important in natural forest situations where the diversity of species is greater.

The most often reported symptom of ash anthracnose is presence of irregular necrotic blotches along the midrib and the margin of leaflets. Necrotic blotches are caused when infection occurs as leaflets expand. Distortion of leaflets can also occur. Leaves that are approaching full size are somewhat resistant, and lesions on them cease to enlarge (2,3,19).

Another characteristic symptom of ash anthracnose is premature drop of leaflets or entire leaves. If this occurs in several successive years, it may lead to dieback (13,19). A small leaf spot phase has also been described as a symptom of ash anthracnose (15).

In California on velvet ash (*F. velutina* Torr.), infected petioles remain on the branches over the winter and may remain attached well into the next season (13). Although perithecia are common on overwintered leaflets and petioles under trees with a prior history of the disease, the importance of ascospores as inoculum in the disease is at present unknown (15). Trees that were infected one year often have severe infection in succeeding years (13).

Differences in susceptibility to anthracnose have been observed within *Fraxinus* (13). Unlike sycamore (*Platanus*) where resistance is known and available (17), there are no ash cultivars with attributes that include resistance to anthracnose (18). Green ash is reported as relatively resistant to ash anthracnose in California (13) but considered susceptible elsewhere (6,19). In another paper in this volume, Redlin and Stack (16) describe studies of *G. fraxini* infection using leaf discs.

Methods and Materials

Observation of symptoms and isolations.—Anthracnose symptoms were observed at several different locations in eastern North Dakota and western Minnesota from May through September beginning in 1982. The sites included green ash trees on boulevards, in field shelterbelts, in an experimental block planting, and in native stands.

At four times during the season isolations were made from each of seven symptom or tissue types: leaf blotch, leaf spot, petiole lesion, overwintered petioles, dead buds, cankers on twigs, and samaras (fruits). For isolations done in May, June, or August

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there were 40-80 isolations for each symptom type; for September isolations, there were 20 of each. Leaflets and green petioles were surface disinfested in 0.25% NaOCl for 15-30 sec followed by a sterile water rinse. Older tissue was surface disinfested in 0.5% NaOCl for 2-4 min. Plates were incubated for 7 days at room temperature (20-24 C) under fluorescent lights. Cultures were maintained on PDA tube slants in a refrigerator at 4 C.

Leaflets with insect feeding injury were collected at five locations where insect feeding was evident. Leaflets were collected to represent three classes: 1) healthy, no insect feeding injury; 2) insect injury but no anthracnose lesions visible; 3) anthracnose small leafspot symptom associated with insect feeding injury. There were 45 isolations in each symptom class. Leaflets were surface-disinfested and pieces plated on PDA as described above.

Comparison of growth and pathogenicity between isolates derived from different sources.—Growth of *Gnomoniella* isolates derived from different symptom or tissue types was compared. Twenty-four isolates were used, four from each of six types. For each isolate tested 7 mm diameter plugs of mycelium and agar taken from the growing margin of a PDA culture was placed on the center of a PDA plate. Four replicate plates of each isolate were incubated at seven different temperatures from 5 to 35 C in the dark for 7 days. Colony size was determined by averaging two perpendicular diameter measurements and daily growth rate calculated.

The same 24 isolates were also compared in laboratory inoculations of green ash leaves. Inoculum of each *G. fraxini* culture was grown and prepared as described below. Incubation chambers were glass petri dishes containing blotter paper and a bent glass rod, sterilized before use. One leaflet or two petioles from greenhouse grown green ash plants, as described below were placed on the glass rod and a 7 mm diam. plug of *G. fraxini* inoculum was placed directly onto the plant surface. For this test, chambers were incubated at 25 C with a 12 hr photoperiod for 6 weeks.

Effect of wounding and site of inoculation on infection.—For this study a single isolate (TA-23) of *G. fraxini* was used. Cultures were grown on PDA and incubated at room temperature with a 12 hr light period. For inoculum, 7 mm diam.plugs were cut from the margin of 14-day-old cultures.

Green ash saplings (2-3 years old) were planted in pots and grown in a greenhouse (25 C day, 18 C night) with supplemental illumination (14 hr photoperiod) supplied by high pressure sodium vapor lamps (Lucalux). Four weeks after bud break, fully expanded leaves were harvested. Leaves were surface disinfested in 0.5% NaOCl for 5 sec then rinsed in sterile water. Wounds were made with a sterile scalpel cutting an X at leaflet midrib, three parallel slashes along the edge of leaflets, or across petioles. The procedures for inoculum production, inoculation and incubation chambers were the same as those for the studies described above.

Treatments of leaflets included: 1) not wounded, not inoculated, 2) inoculated but not wounded, 3) wounded at midrib, 4) wounded and inoculated at midrib, 5) wounded at edge, 6) wounded and inoculated at edge, 7) inoculated at edge. Treatments of petioles were: 1) wounded only, 2) inoculated only, 3) wounded and inoculated. All leaves and petioles were incubated at three temperatures, 16, 20 and 25 C without light. There were six replicate chambers for each treatment combination. After preparation, chambers were wrapped in aluminum foil and placed in incubators at 16, 20 and 25 C. After 6 weeks the degree of infection was determined. Reisolations from representative samples were made on PDA.

Greenhouse inoculations.—Inoculum of isolate TA-23 was prepared by blending four 3-week-old plate cultures in 10 ml of sterile distilled water. Inoculations were done in the greenhouse using the method of Black and Neely (1) in which a spore suspension was brushed onto both leaf surfaces of 25 leaflets. Sterile distilled water was applied on five leaflets as a control.

A similar experiment was done three times, using a higher inoculum concentration applied to both leaf surfaces with an atomizer. In each experiment 105 leaflets were inoculated. Sterile distilled water was sprayed on the same number of leaflets as a control. Inoculated and control trees were then placed in a humidity chamber (9) with an average temperature of 21 C, at 98% RH for 4 weeks.

Survey.—Severe outbreaks of ash anthracnose occurred during the spring of 1982 in Grand Forks, ND on boulevards planted in a monoculture of green ash. According to local sources (J. Staley, Grand Forks city forester, personal communication) trees in this area had suffered severe anthracnose in several previous years. Data collected from 1123 boulevard trees at 24 different sites included the number of trees infected, intensity of infection, average height of trees, and canopy closure. Isolations from these trees showed *G. fraxini* in association with the various symptoms.

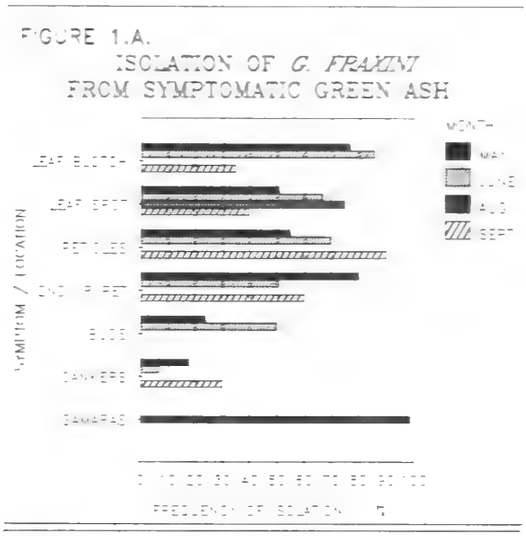
Based on this survey a more detailed study was made of six of the sites. Each site contained about 50 trees. Each tree was rated for presence or absence of petiole infection, twig blight and cankers, defoliation, foliar symptoms, sex, canopy type and the amount of foliage infected in the upper, middle and lower thirds of the tree crown. Sites were evaluated for clustering of infected trees in disease focusing Madden's 'Ordinary Runs' test for randomness of infected plants (10).

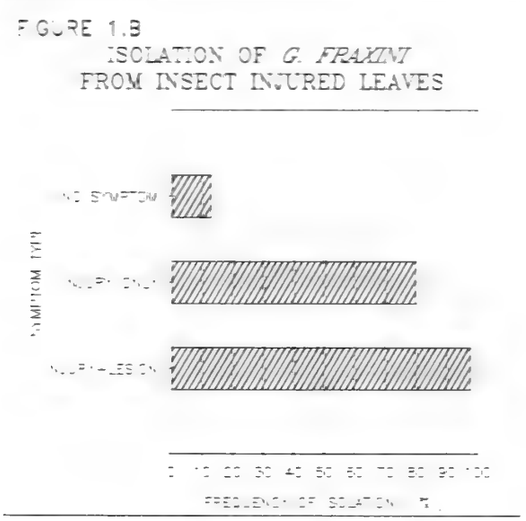
This analysis tests for clustering of infected plants based on the null hypothesis for randomness and has less than 5% misclassification. To apply this test, each site was sub-divided into six sub-plots or stands containing 7-9 trees. Each tree had already been separately scored for disease severity. Chi-square tests were used to determine differences in infection between male and female trees and between canopy closure types.

Results

Observation of symptoms and isolations.—The year 1982 was favorable for development of anthracnose and all seven symptom types were seen. From isolations from these symptoms, *G. fraxini* was found associated with all symptom and tissue types and at every time of the season but not with the same frequency. The frequency of isolation of *G. fraxini* from each of the symptom types and times is shown in fig. 1A. It was most frequently isolated from samaras (fruits), leaves and petioles, less frequently from buds and least often from cankers.

Leaf blotch was the most obvious symptom in early spring and *G. fraxinii* recovery was 77% of 80 isolations in May and 84% of 72 isolations in June. Recovery of *G. fraxini* from petioles and samaras was high in spring and summer but declined in September (fig. 1A). Isolation from buds and cankers was at much lower frequencies. *Gnomoniella fraxini* was consistently associated with the typical ash anthracnose symptoms (such as leaf blotch) but also symptoms not previously described, such as fruits and petioles.



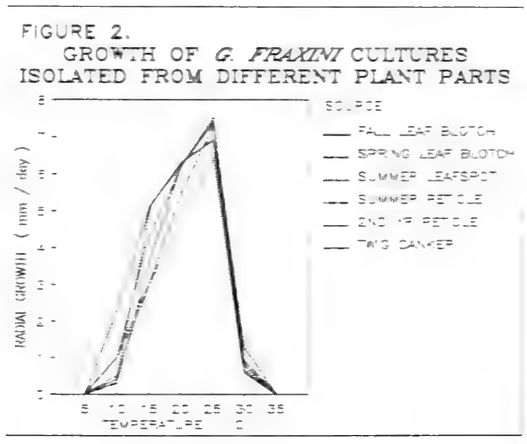


Cultures of *G. fraxini* derived from all sources were similar in appearance and were easily recognized by characteristic color and form (15). Colonies on PDA were initially white but soon became purplish-red and displayed a concentric zonation. After about 10 days, conidiomata appeared producing slimy, pinkish masses of conidia. Acervuli on inoculated leaves were identical to those found on naturally infected plants.

Most of the trees in the severely diseased sites (see below) had petioles from the previous year still attached to twigs in the spring. These petioles persisted even after the new flush of growth had begun and were nearly always covered with acervuli of the *Discula* stage of *G. fraxini* (fig. 6).

Association of anthracnose with insect injury.—Insects injuring leaves were identified as ash plant bug, *Neoborus anoenus* Reuter. (Identity was confirmed by E. W. Balsbaugh, Dept. of Entomology, North Dakota State University.) The stippled injury observed was typical of feeding by this type of insect (8). *Gnononiella fraxini* was isolated from a small proportion of apparently healthy leaves but from the majority (80%) of insect injured leaves, as well as from leaves with anthracnose lesions (fig. 1B).

Growth and pathogenicity tests between isolates.—Growth rates for the isolates from the six symptom or tissue types all followed the same pattern (fig. 2). Fastest growth of all isolates was at 25 C with an optimum range of 15-25 C. Growth was sharply reduced at 10 and 30 C. There was no growth at 5 or 35 C within 14 days. When those plates were placed at 25 C, all isolates from 5 C grew but the isolates from 35 C did not grow.



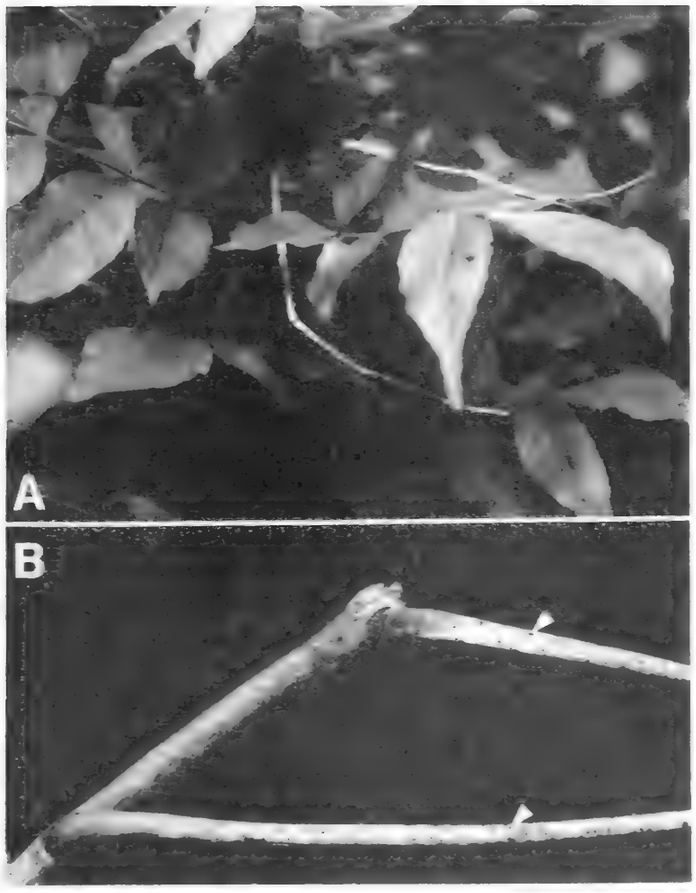
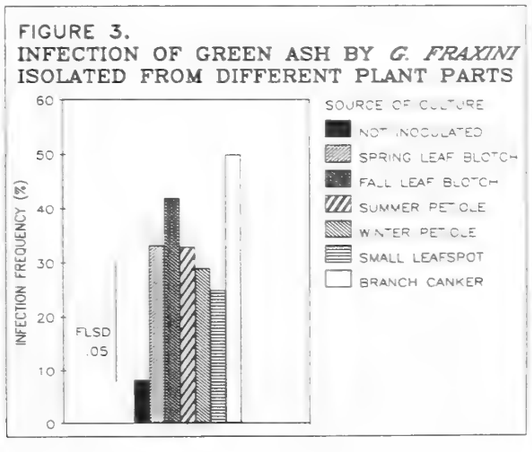


Figure 6.—Infected overwintered petioles.

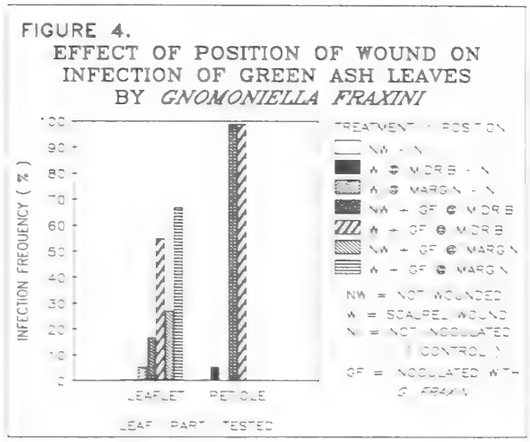
A. Shown in mid-June after leaves have expanded. The proximity of petioles to current leaves serves as an ideally placed souce of primary inoculum and new leaves are already infected. Terimnal bud of shoot with overwintered petioles has been killed by earlier infection.

B. Petioles that are clinging to the previous year's growth on green ash before budbreak in the spring. The small, black, round to ellipitcal specks (arrows) are *D. fraxinea* acervuli.

When this set of isolates were compared for pathogenicity, all caused some infection. Isolates from the six sources did not differ significantly from each other in disease-causing ability (fig. 3).



Effect of wounding and inoculation site on infection.—Wounding significantly increased infection of leaflets (fig. 4). The wounded leaflets inoculated at midrib and margin had 56% and 67% infection while the corresponding inoculated but nonwounded treatments inoculated had only 17% and 28% infection, respectively. Both nonwounded and wounded petioles showed 100% infection when inoculated with *G. fraxini*. The symptom of infection was necrosis of the area around the inoculation site. Incidence of infection of leaflets and petioles was not influenced by the temperatures (16, 20, 25 C) used in these experiments. *Gnomoniella fraxini* was reisolated from petioles or leaflets showing symptoms.



The above experiment was repeated but using a mixture of isolates of *G. fraxini*. Results were similar to the previous experiment. Wounding followed by *G. fraxini* inoculation resulted in significantly greater infection of leaflets inoculated at midrib or margin (25%, 40%, respectively) than the corresponding nonwounded inoculated controls (5%, 9%, respectively). Infection of inoculated, wounded petioles was 35%. Noninoculated controls remained healthy.

Greenhouse inoculations.—In one experiment all 25 of the leaflets brush-inoculated with *G. fraxini* showed blotch symptoms after four weeks. Three control leaflets also became necrotic but *G. fraxini* was not isolated from them. *Gnomoniella fraxini* was reisolated from inoculated leaflets showing symptoms. In three additional experiments where plants having spray-inoculated leaves were incubated in the humidity chamber, 81%, 85% and 86% of leaflets showed necrosis in experiments 2, 3 and 4, respectively.

Survey.—Green ash monoculture on boulevards is widespread in the city of Grand Forks, ND. Of the 24 sites surveyed, four had severe anthracnose, four were moderately affected and sixteen were slightly affected. Severity of anthracnose appeared to be related to degree of canopy closure (fig. 5A). Of the sites with severe infection, three of the four had a closed canopy along the boulevard but not across it. In moderately diseased sites, one of the four had a closed canopy. All of the sites with slight symptoms had open canopies.

FIGURE 5.A
EFFECT OF CANOPY CLOSURE ON SEVERITY OF ASH ANTHRACNOSE

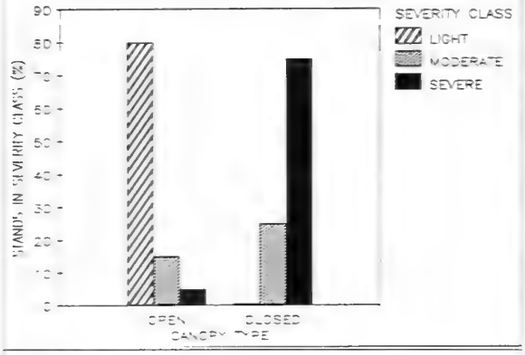


FIGURE 5.B.
SEVERITY OF ASH ANTHRACNOSE ON MALE AND FEMALE GREEN ASH TREES

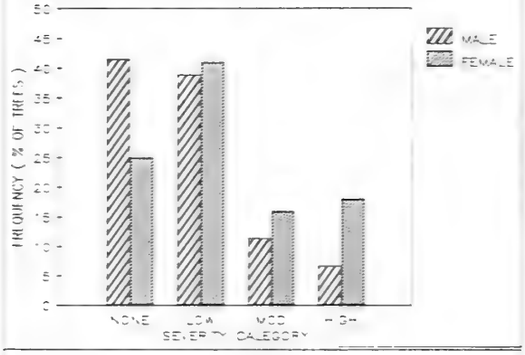
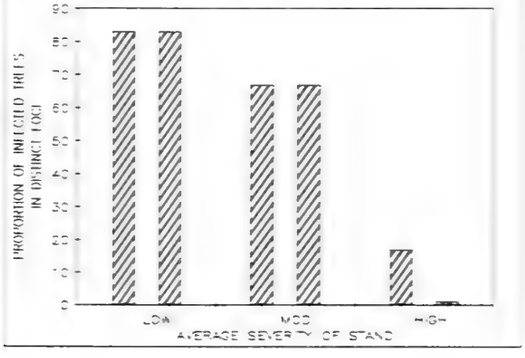


FIGURE 5.C.
OCCURRENCE OF ANTHRACNOSE FOCI IN STANDS DIFFERING IN DISEASE SEVERITY



Of six intensively studied sites, there were two each in high, moderate and low severity classes. In nearly all cases the top third of each tree had the least disease and the lower third the most. At five of the six intensively studied sites there were about equal numbers of male and female trees, appearing to be more or less randomly distributed. The trees were sorted individually according to sex and anthracnose severity (none, slight, moderate or severe) and the frequencies compared by chi-square. There was a significant relationship between sex of the tree and disease severity (chi-square (4 df) = 94., $p < .01$). A greater proportion of female trees were in the moderate and severe categories and fewer were free of symptoms (fig. 5B).

To determine if the severely infected trees occurred randomly or were grouped in foci, we applied the cluster analysis technique of Madden et al. (10). Of the 36 subplot stands, 19 showed clustering of infections into foci. The subplot stands showing presence of disease foci were not uniformly distributed among the sites; however, most were in the slightly or moderately diseased sites (fig. 5C).

These green ash monocultures in Grand Forks, ND were resurveyed each year from 1982 through 1988. Of those seven seasons, there were two when anthracnose was severe (1982, 1984), and two when it was moderate (1983, 1986). In the remaining three seasons, there was little or no anthracnose in these plantings. Trees which had exhibited moderate-to-severe anthracnose during the period 1982-1986 showed dieback of branches and other symptoms of general decline in 1987 and 1988.

Discussion

The high proportion of *G. fraxini* isolated from leaf parts in early summer indicates this fungus is a primary pathogen. Later in the season, many facultative and secondary parasites colonize senescing leaves. As those fungi increase, *G. fraxini* isolated is proportionately less of the the total. *Gnomoniella fraxini* does retain possession of colonized leaves into the following spring; perithecia are abundant on leaves under anthracnose-affected trees in May (15). On petioles and fruits *G. fraxini* remained the predominant fungus even well into the second year. These are covered with acervuli and are ideally placed to act as sources of primary inoculum. The lower rate of recovery from buds is probably because many buds that do not grow in spring are not diseased but frost-killed. Cankers caused by *G. fraxini* probably are not a particularly important aspect of ash anthracnose.

All isolates of *G. fraxini* had an optimum temperature for growth of 25 C. This agrees with studies by Ogawa et al. (18) who found best growth at 20-27 C. This is different from the oak and sycamore anthracnose fungi whose growth optima are 12-20 C (11). Wounding before inoculation increased infection over treatments where tissue was not wounded. This is similar to results of Neely and Himelick (12) who wounded leaves before artificial inoculation to achieve a high percentage of infection. In later studies Redlin and Stack (16) also found wounding necessary for high levels of infection.

While *G. fraxini* should be considered a primary pathogen, infection does appear to be enhanced by wounding. This is also the case with both sycamore and oak anthracnose (12). Our observation of association between *G. fraxini* and feeding by the ash plant bug suggests this injury may be an important site of infection in nature.

The two surveys showed that disease incidence and severity can be very high in monocultures of green ash, agreeing with the results of Ogawa (13) on velvet ash. Incidence and severity also appear to be related to the density of the canopy. Sites with a closed canopy had more severely infected trees than those with open canopies.

Anthracnose was most severe in the lower third of the tree crown. This agrees with findings in California (3) and with results of Neely in Illinois for sycamore anthracnose (11).

Persistence of infected petioles over winter was a symptom consistently associated with anthracnose-infected trees. Most of the trees in the severely diseased sites had petioles from the previous year still attached to twigs in the spring.

The difference in infection between male and female trees is puzzling. While there may be some genotypic difference, a more likely explanation would relate to the difference in influorescence structure and persistence. Green ash seed do not mature until fall and many persist into winter, as do the peduncles and other supporting structures (5). In our observations, old seed stalks were often present well into the next season; thus, female trees have an additional source of inoculum besides overwintered leaf petioles. If seed stalks are colonized by *G. fraxini* at levels comparable to petioles, this added inoculum might well explain the higher disease levels in female trees.

Diseased trees appeared to be in foci on the slight and moderately infected sites but not at heavily infected sites. It may be that the severe sites were single large foci, a circumstance our statistical test would not detect. At many locations severely and moderately infected trees were located adjacent to slightly affected or healthy ones. These healthy trees could be escapes from infection, especially in areas with an open canopy but it seems likely that a genetic component is also involved since most of the sites were planted with seed-grown stock which exhibits wide variation in many characters of form, development and foliation.

Variation in anthracnose susceptibility in open-pollinated seedling populations has been reported for sycamore and walnut, as well as for many other foliage diseases. The individual trees showing high or low disease severities in 1982 were re-examined in succeeding years. They showed the same reaction from year to year. Individual healthy trees in severely diseased sites are likely candidates for further testing and possible introduction as resistant clones. While this study was done on urban boulevard sites, ash planted in farmstead windbreaks or field shelterbelts are in the same kind of linear monocultures. These findings can be expected to apply in those situations as well.

More research is needed on anthracnose diseases in general and on ash anthracnose in particular. Specific areas of additional research needed are the infection process, resistance mechanisms and additional information on epidemiology. Ash anthracnose can be expected to increase in importance with increased planting of green ash.

Acknowledgements

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Literature Cited

1. Black W. M., Neely, D. 1978. Effects of temperature, free moisture, and relative humidity on the occurrence of walnut anthracnose. Phytopathology 68:1054-1056.
2. Carter, J. C. 1975. Diseases of Midwest Trees. Univ. Illinois Special Publ. 35. 168 p.
3. Coe, D. M., Wagener, W. W. 1949. Ash anthracnose appears in California. Plant Dis. Rep. 33:232.
4. Conners, I. L. 1967. An Annotated Index of Plant Diseases in Canada and Fungi Recorded on Plants in Alaska, Canada and Greenland. Canada Dept. Agric. Publ. 1251. 381 p.
5. Fowells, H. A. 1965. Silvics of Forest Trees of the United States. USDA Forest Serv. Agric. Handb. 271. 762 p.
6. Hepting, G. H. 1971. Diseases of Forest and Shade Trees of the United States. USDA Forest Serv. Agric. Handb. 386. 659 p.
7. Hoag, D. G. 1965. Trees and Shrubs for the Northern Plains. N. D. Inst. Regional Studies. Fargo, ND. 376 p.
8. Johnson, W. T., Lyon, H. H. 1976. Insects that Feed on Trees and Shrubs: An Illustrated Practical Guide. Cornell Univ. Press, Ithaca, NY. 464 p.
9. Krupinsky, J. M., Scharen, A.L. 1983. A high humidity incubation chamber for foliar pathogens. Plant Dis. 67:84-86.
10. Madden, L. V., Louie, R., Abt, J. J., Knoke, J. K. 1982. Evaluation of tests for randomness of infected plants. Phytopathology 72:195-198.

11. Neely, D., Himelick, E.B. 1963. Temperature and sycamore anthracnose severity. Plant Dis. Rep. 47:171-175.
12. Neely, D., Himelick, E.B. 1967. Characteristics and nomenclature of the oak anthracnose fungus. Phytopathology 57:1230-1236.
13. Ogawa, J. M., Bose, E.,Manji, B. T., Peterson, L. J. 1977. Life cycle and chemical control of Modesto tree anthracnose fungus. Phytopathology 57:1230-1236.
14. Redlin, S. C., Stack, R.W. 1986. Effect of temperature on germination of conidia of *Gloeosporium aridum*. Proc. North Dakota Acad. Sci. 40:63.
15. Redlin, S. C., R. W. Stack, R.W. 1988. *Gnomoniella fraxini* sp. nov., teleomorph of the ash anthracnose fungus and its connection to *Discula fraxinea* comb. nov. Mycotaxon 32:175-198.
16. Redlin, S. C., Stack, R.W. 1990. An in vitro technique to evaluate infection of green ash by *Gnomoniella fraxini*. p. 137-145. In: W. Merrill and M. Ostry (eds.). Recent Research on Foliage Diseases. USDA For. Serv. Gen. Tech. Rep. WO-56, 145 p.
17. Santamour, F. S., Jr. 1976. Resistance to sycamore anthracnose disease in hybrid *Platanus*. Plant Dis. Rep. 60:161-162.
18. Santamour, F. S.Jr., McArdle, A.J. 1983. Checklist of cultivars of North American ash (*Fraxinus*) species. J. Arbor. 9:271-276.
19. Sinclair, W. A., Lyon, H.H., Johnson, T.W. 1987. Diseases of Trees and Shrubs. Cornell Univ. Press, Ithaca, NY. 574 p.
20. U.S. Dept. Agric. 1960. Index of Plant Diseases in the Unitd States. USDA Agric. Handb. 165. 531 p.

An In Vitro Technique to Evaluate Infection of *Fraxinus Pennsylvanica* by *Gnomoniella Fraxini*[1]

Scott C. Redlin[2,3] and Robert W. Stack[2]

Abstract.—A reliable laboratory method of evaluating ash anthracnose infection of green ash leaf discs is described. The method was useful in studying the effects of wounding, inoculum concentration, temperature and leaf age on infection. Other methods of controlled inoculation were slow to produce infection. This method shows promise as a method of screening *Fraxinus* for resistance to anthracnose.

Introduction

Ash anthracnose and its causal fungus.—Ash anthracnose, caused by the parasitic fungus *Gnomoniella fraxini* Redlin & Stack (19), occurs on most *Fraxinus* spp. native to North America (22) and may cause extensive defoliation of green ash (*Fraxinus pennsylvanica* Marsh.). The causal fungus had long been known as *Gloeosporium aridum* Ellis & Holway. Observations suggest susceptibility to ash anthracnose varies among individual trees. Snyder (23) observed disease differences in green ash trees grown from seed which she interpreted as due to genetic differences. Even though there are many cultivars of *F. pennsylvanica,* no resistant cultivars have been marketed in the commercial nursery trade (20). Several techniques used to assay other species of trees for resistance to leaf diseases were unsatisfactory when used to assay *Fraxinus* spp. for resistance to anthracnose. Symptoms of successful infections were slow to appear and results of comparable tests were inconsistent. A better technique for screening ash for resistance to anthracnose was needed and this paper reports results of such a technique using excised leaf discs for the procedure.

Methods utilized to study other anthracnose diseases.—Controlled inoculations with anthracnose fungi have been done on plants grown in the greenhouse. Conidial suspensions are sprayed on or applied with a brush to unwounded leaf surfaces and plants are placed in mist chambers or enclosed within polyethylene bags (2,23). Neely and Himelick (12) used a slightly different technique to obtain infection. They burn-wounded leaves of oak and sycamore prior to inoculation. Infection of oak and sycamore seedlings was evaluated approximately 3 to 4 weeks after incubation at greenhouse temperatures (12). Snyder (23) obtained infection on ash leaves contained in petri plates by inoculating with mycelium and agar. She also obtained infection of green ash seedlings after inoculating with a conidial suspension and incubating intact plants in a mist tent for 6 weeks. Walnut leaf age was quantified and its relationship to susceptibility to anthracnose infection in walnut was investigated (4).

Uses of disc testing.—Methods of detached leaf culture (28) have been replaced by the use of leaf discs for examining many factors that affect leaves of cultivated plants. Disc testing has been used to evaluate *Populus* for resistance to *Marssonina* (25) and *Melampsora* (21) and *Malus* for resistance to *Venturia* (1). Tropical tree genera studied in a similar manner include *Coffea/Hemileia* (9) and *Hevea/Microcyclus* (3). Fungal diseases of lettuce (8,11), onion (5) and cabbage (6) have also been studied with the leaf disc method.

After removal from leaves, discs have been handled in several ways. They include placement on moistened filter papers in chamber (7), directly on agar sufaces (10) or placement within agar wells cut the same diameter as the disc (14,17,21,25). The latter method seems to have been most successful with plant pathogens.

In the studies reported here, the agar disc method of Spiers (25) was combined with the wounding method of Neely and Himelick (12) to study ash anthracnose. A preliminary report regarding the study of ash anthracnose infection using the leaf disc testing method has been published (17).

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Methods

To provide a constant supply of young leaves, supplemental lighting was used to provide long day (14 h) conditions in the greenhouse, causing ash seedling trees to break dormancy and commence shoot growth (previous cold storage had satisfied the dormancy requirement). Continued long days provided by supplemental lighting in combination with removal of the leaves from the first growth flush, promoted secondary flushes of growth.

Leaf discs.—Healthy leaves were collected from 2- or 3-year-old ash seedlings grown in the greenhouse. Leaf discs, 18 mm diam, were excised from the leaves with a #10 cork borer. Discs were stored between damp paper towels at 4 C until used in experiments, within 2 days. Just prior to use, discs were surface disinfested in 0.25% sodium hypochlorite for 45 sec, twice rinsed for 1 min with sterile distilled water and blotted dry with sterile paper towels.

Plastic petri dishes (100 x 15 mm) were prepared containing a 4-mm-thick layer of 2% water agar. Wells 18 mm in diam. were punched in the agar of each plate with a sterile cork borer and the agar plugs removed. A surface-disinfested leaf disc was inserted abaxial surface uppermost into each well with a sterile forceps (fig. 1).

Preparation of inoculum.—*Gnomoniella fraxini* isolates were maintained on potato dextrose agar (PDA) slants at 4 C. Tube slants were removed from cold storage and stored at 20-24 C for 3 to 4 days before isolates were transferred to PDA plates. Resultant cultures were grown at room temperature 20-24 C ca 30 cm below fluorescent lights (F40 WW + F40 CW) on a 12 h photoperiod for approximately 30 days.

Conidial inoculum was prepared by removing pieces of agar with abundant conidiomata and placing them in tubes containing 5 ml of sterile distilled water. Following vigorous shaking by hand, conidia were uniformly suspended by swirling for several min on a Vortex mixer. The concentration was estimated with a hemocytometer. Following the experiments on inoculum conentration described below, a standard concentration of 13,000 conidia/10 µl droplet was used in all other experiments.

Inoculation and incubation of leaf discs.—Inoculation of leaf discs was done by removing 10 µl of conidial suspension with a fixed stroke micropipette and placing it on the center of nonwounded or wounded leaf discs. A 10 µl droplet of sterile distilled water was placed on the center of each noninoculated disc as a control.

Plates were sealed and incubated at the temperatures specified in each experiment. Plates incubated in the dark were wrapped in aluminum foil. Plates containing discs incubated in the light were placed approximately 30 cm below a fixture containing two 40 w tubes on a 12 h photoperiod at 20 to 24 C.

Some of the following experiments were done to establish standard procedures. Where not otherwise specified in the experimental methods described below, leaf discs were 18 mm in diam., cut from 20-day-old leaves, burn-wounded (5-6 mm diam. region of necrosis), incubated at room temperature (20 to 24 C) and inoculated with a single 10 µl droplet of spore suspension containing 13,000 conidia. In all experiments, noninoculated control discs remained healthy.

Wounding.—Wounding of leaf discs was done in three ways, abrading with carborundum applied with a sterile cotton swab; cutting through the leaf with a sterile scalpel; and burning with a heated rod. Abraded areas were examined under the dissecting microscope for evidence of infection. On cut discs, the width of necrotic tissue was measured at right angles to the cut. Burn-wounding was done by heating a 4 mm diam. aluminum rod with an alcohol burner and pressing it onto the upper surface of leaf discs for approximately 1 sec. Within 30 min of burn-wounding, a necrotic area about 5 to 6 mm diam. with a distinct margin became apparent on leaf discs. In the following 24 to 48 h, the color of the burned tissue changed from dark green to a light tan but further size expansion did not occur. This area of necrosis will be referred to as a "burn wound." A leaf disc was considered infected if, after burn-wounding and inoculation with *G. fraxini*, it had an additional ring-like zone of necrosis. This area of collapse due to pathogenic growth of the fungus will be referred to as a "lesion". After 9 days, burn wounds and lesions on leaf discs were measured at 15X with a dissecting microscope equipped with an eyepiece micrometer. Two diameter measurements were made across each lesion at right angles and averaged. Following this experiment on wounding method, the burn wound described here was adopted as the standard technique and was used in all subsequent studies.

Effect of inoculum concentration on infection of leaf discs.—Green ash leaf discs were placed into wells of water agar plates and burn-wounded discs were inoculated with concentrations of 123,500, 13,000, 5,250 and 625 conidia/10 µl droplet of suspension. The experiment was done three times.

Leaf age and its effect on infection.—The generalized growth curve of leaf expansion (fig. 2G) was used as a guide for sampling leaves by leaf disc tests. Earlier experiments utilized discs from 10-, 20- and 30-day old leaves. Later studies examined closer intervals along the developmental curve. The points of removing leaves for discs are indicated by arrows in fig. 2G.

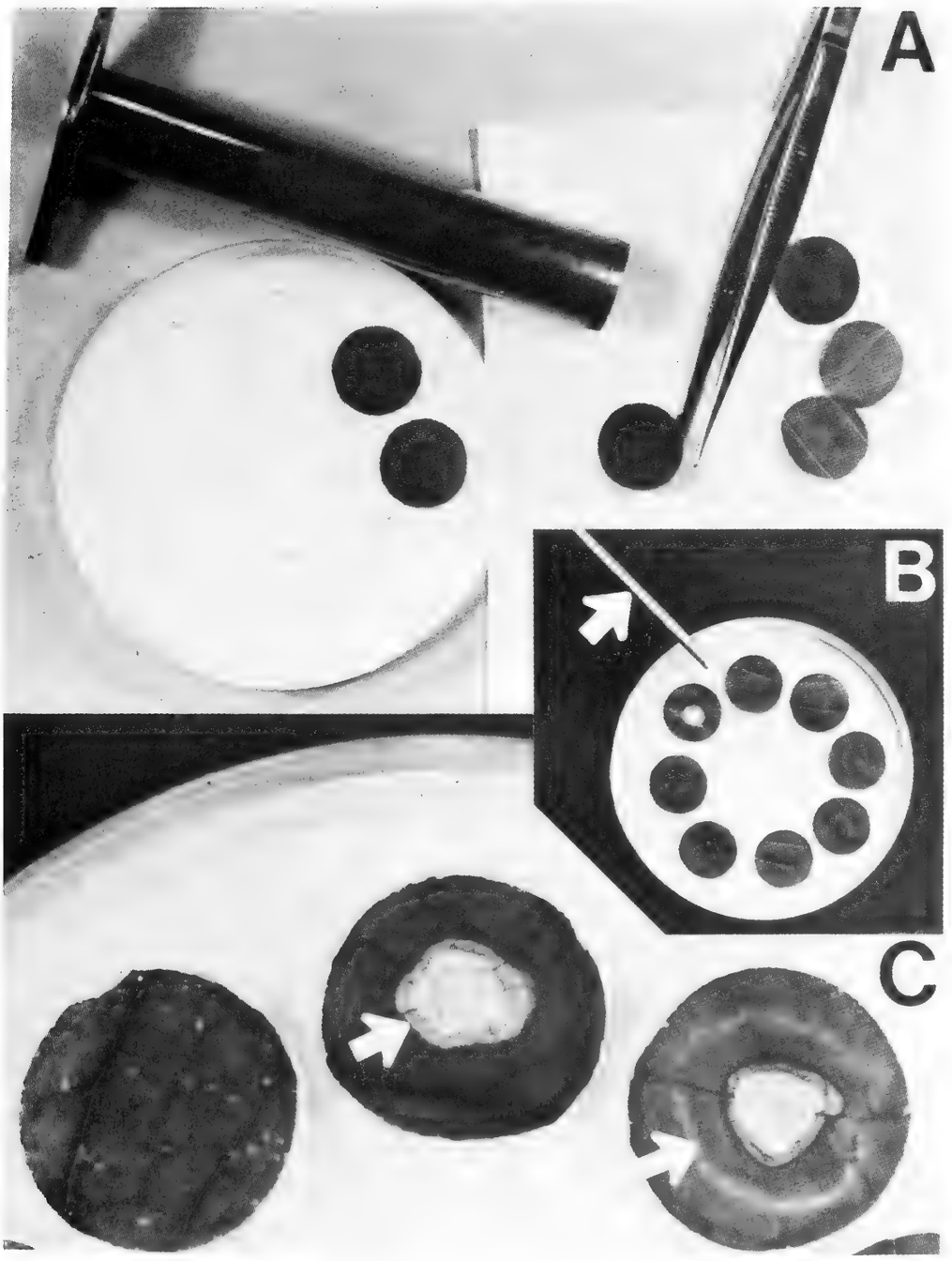

Figure 1.—In vitro technioque used to study infection of green ash by *Gnomoniella fraxini*.

A. Plastic petri dish (100 mm diam.) containing 2% water agar (4 mm deep) with wells cut using a sterile cork borer (18 mm diam.) and leaf discs of corresponding size being placed into wells with a sterile forceps.

B. Arrangement of ash leaf discs in wells in water agar. Arrow indicates 4 mm diam. aluminum rod the tip of which was heated and touched to leaf discs for wounding treatment. A burn-wounded leaf disc is shown below the rod tip.

C. Green ash leaf discs nine days after inoculation. Nonwounded disc (L) remained healthy with normal green color (small spots are water droplets on the disc surface). Wounded noninoculated disc (C) shows necrotic tissue (arrow) resulting from original burn wound. Wounded disc inoculated with *G. fraxini*. (R) shows lesion developing beyond original burn wound. Arrow indicates lesion margin surrounded by chlorotic ring.

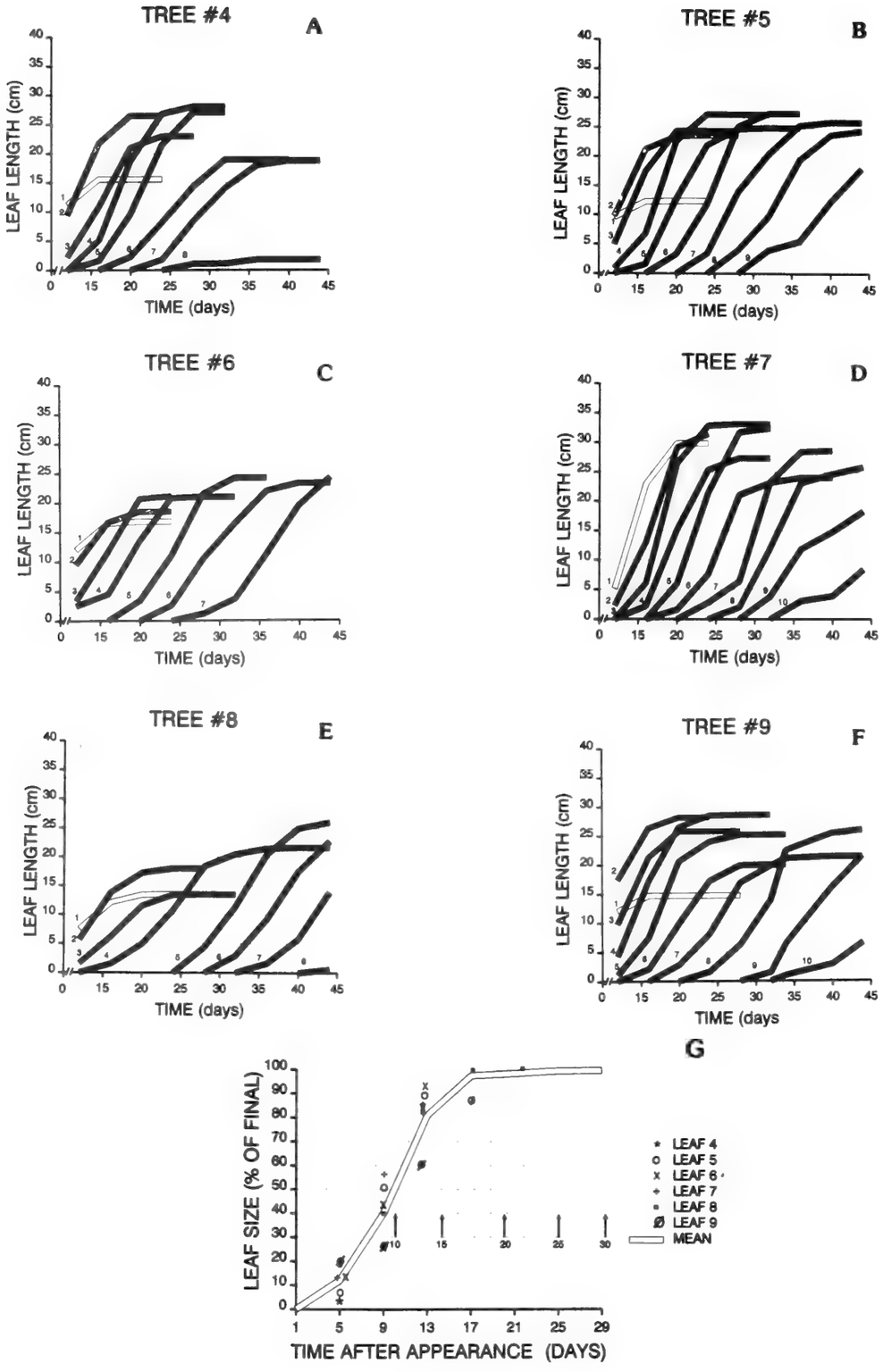

Figure 2.—A-G. Leaf development of six green ash seedlings grown in the greenhouse.

A-F. Size (leaf length) of individual leaves at up to 10 nodes. Length of one leaf per node measured from axillary leaf bud to tip of terminal leaflet. Leaves numbered in order of appearance from bottom to top of shoot. Growth curves of individual leaves based on a greenhouse grown green ash seedling tree. Time in days after bringing potted dormant tree into a 20-25 C greenhouse.

G. General growth curve for leaves 4-9 from tree #5. Leaf development expressed as percent of full expansion. Points for individual leaves as indicated. Line connects means. Arrows indicate times at which leaves were removed from trees for preparation of leaf discs in four subsequent experiments. Arrows at 10, 20 and 30 days indicate sampling times for the first two experiments. Arrows at 15, 20 20 days indicate sampling times for the second two experiments.

In two experiments, leaves approximately 10, 20 and 30 days old were collected and leaf discs were prepared for testing. Nine wells were made per water agar plate and three leaf discs of each age class were placed into each plate. The treatments given to each age class were 1) nonwounded (NW), noninoculated (NI), 2) wounded, noninoculated (NI) and 3) wounded + inoculated with a leaf spot isolate of *G. fraxini*. In two additional experiments the leaf discs were removed from leaves aged 15, 20 and 25 days.

Effect of temperature on lesion size.—The effect of temperature on infection of green ash leaf discs was studied in two experiments using a single isolate of *G. fraxini*. Each plate contained eight leaf discs (four pairs) from one tree. From each pair, one disc was wounded and not inoculated and the other was wounded and inoculated. In the first experiment, dishes with leaf discs were incubated in the dark at 5, 10, 15, 20, 25, 30 and 35 C. In the second experiment, all the temperatures were the same except that 35 C was omitted.

Effect of sources of isolates.—The size of lesions on green ash leaf discs inoculated with *G. fraxini* from four sources was studied in two experiments. Each plate contained eight leaf discs (four pairs) from one tree. From each pair, one disc was wounded and not inoculated and the other was wounded and inoculated. The sources of the isolates were leaf spots, single ascospores, acervuli and cankers. Each pair of leaf discs was inoculated with one of 12 isolates. A set of three replicate plates was wrapped together in heavy duty aluminum foil and incubated in the dark at 16, 22 or 28 C. The experiment was done twice.

Experimental design.—Dishes to be treated as a block in each experiment were wrapped together in aluminum foil or grouped together under flourescent lights during incubation. A randomized complete block design with three, four or six replicate blocks was used for the layout of all experiments. Experiments were analyzed by analysis of variance (ANOVA) and the FLSD statistic was used for treatment comparisons (27).

Results

Leaf development.—Most leaves of green ash required approximately 21 days from their initial appearance to full expansion. The typical patterns of development for leaves of six green ash seedling trees are presented in figure 2A-F. Growth curves for individual leaves were generally sigmoidal in form. Leaves developed sequentially and most leaves were 20 to 28 cm in length at maturity. To produce a generalized growth curve for leaves, the curve for each leaf was shifted and superimposed to begin at day one (fig. 2G). Leaves one through three were not included because they were already too large when measurements were begun. In general, leaves were about 50% expanded after 10 days and 90% expanded by 15-16 days.

Effect of leaf age on infection of leaf discs.—In the first two experiments, leaves 10, 20 and 30 days old were collected from the greenhouse-grown green ash seedlings. In the first experiment all of the wounded inoculated discs had significantly larger lesions than any of the noninoculated controls. Lesion size was significantly greater on the youngest (10-day old) than on the older (20- or 30-day old) discs. Lesions on burn-wounded and inoculated 20-day old discs were not significantly different in size than those on burn-wounded and inoculated 30-day old discs.

There were significantly larger lesions on the wounded inoculated 10-day old discs in the second experiment. In the other age classes, the wounded inoculated treatments were not significantly different than the wounded noninoculated controls. The results of the two experiments were averaged and the means are presented in figure 3C.

In two additional experiments, leaves 15-, 20- and 25-days-old were collected for use in leaf disc tests. In the third experiment, inoculated discs had significantly larger lesions than noninoculated burn wounds. Burn-wounded and inoculated discs from 20-day-old leaves had significantly larger lesions than similarly treated discs from either 15- or 25-day-old leaves. Wounded but noninoculated discs of all age classes were not significantly different. In the fourth experiment, lesions on 15- and 20-day-old wounded and inoculated leaf discs were significantly larger than the 25-day-old wounded and inoculated leaf discs. Wounded and inoculated discs had significantly larger lesions than their respective controls. Leaf discs of 20 days old were used in further experimentation. The results of the third and fourth experiment were averaged and the means are presented as figure 3D.

Effect of wounding and inoculation on leaf discs.—In all three experiments testing the type of wounding treatment, significantly larger lesions developed from inoculated burn wounds than the others (fig. 3A). Discs with inoculated cut wounds had little if any lesion expansion and lesions were not significantly larger than the cut wounds themselves. Unwounded discs remained healthy for up to 60 days.

Effect of inoculum concentration on infection of leaf discs.—Three experiments were done to test the effect of different inoculum concentrations on infection by *G. fraxini*; All experiments used burn-wounded discs for both inoculated and control treatments. In all three experiments, the inoculated discs

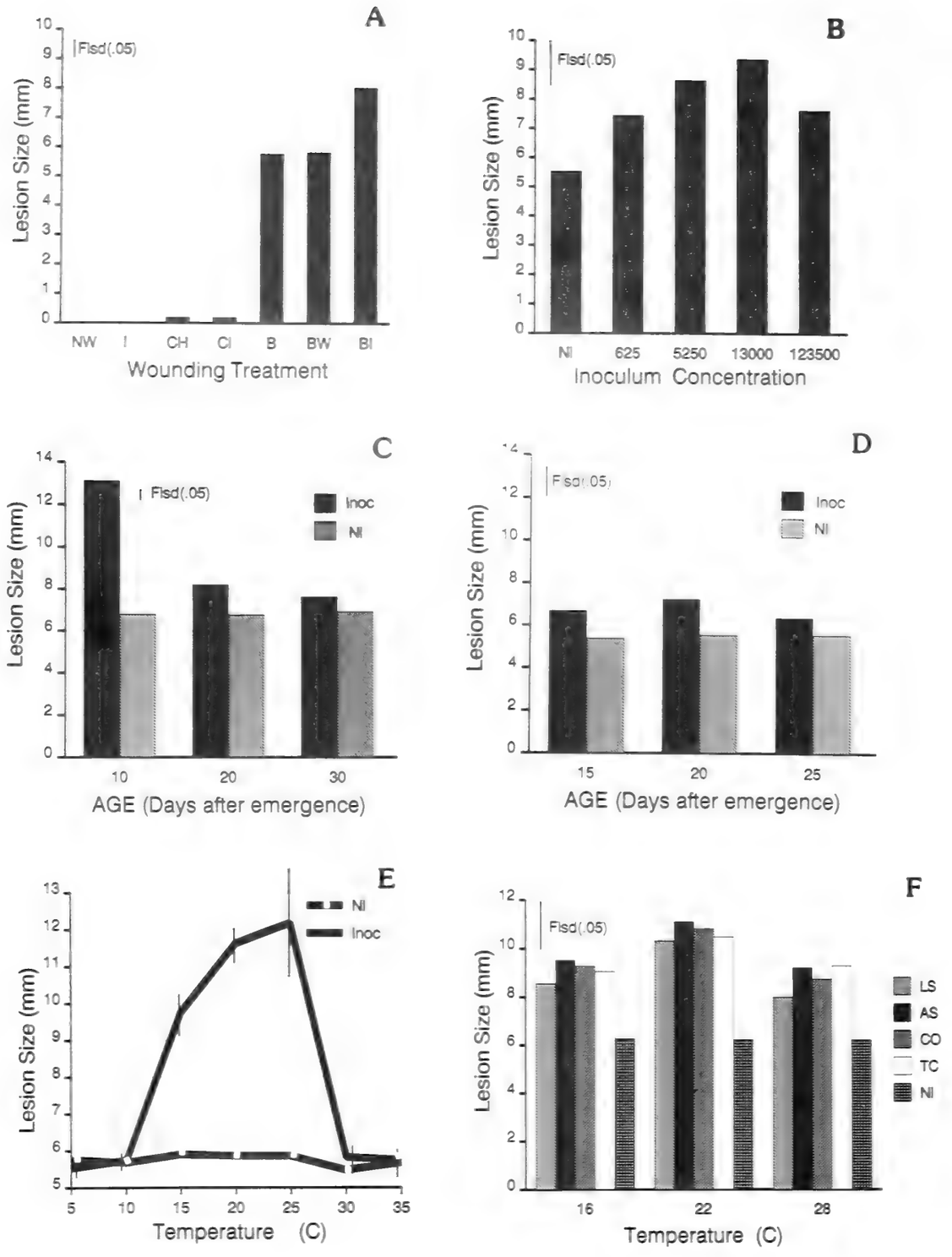

Figure 3.—Effect of treatments and experimental conditions on size of lesions on green ash leaf discs inoculated with *G. fraxini*. A. Effect of type of wounding. Means of three experiments. Treatments were NW = Nonwounded with a 10 µl droplet of sterile distilled water, NI = Nonwounded, inoculated with *G. fraxini*, CH = Cut-wounded and a 10 µl droplet of sterile distilled water applied, CI = Cut-wounded and inoculated with *G. fraxini*, B = Burn-wounded, not inoculated, BW = Burn-wounded and a 10 µl droplet of sterile distilled water applied. BI = Burn-wounded and inoculated with G. fraxini. In fig. 3B-F, the treatment labelled NI corresponds to treatment here called BW.

B. Effect of inoculum concentration (conidia per leaf disc) on size of lesions on green ash leaf discs inoculated with *G. fraxini*. Means of three experiments.

C. Effect of leaf age on size of lesions on green ash leaf discs noculated with *G. fraxini*. Means of two experiments using leaves aged 10, 20 and 30 days after emergence. Leaves at all stages collected at one time.

D. Effect of leaf age on size of lesions on green ash leaf discs inoculated with *G. fraxini*. Means of two experiments using leaves aged 15, 20 and 25 days after emergence. All leaves removed at one time.

E. Effect of temperature on size of lesion on green ash leaf discs inoculated with *G. fraxini*. This figure shows the results of two experiments plotted together. Each experiment consists of an inoculated treatment and a noninoculated control, both burn-wounded.

F. Effect of temperature on lesion size on green ash leaf discs inoculated with *G. fraxini* from four sources. Means of two experiments. Legend indicates source of cultures: LS = Leaf spots, AS = Ascospores, CO = Conidia, TC = Twig cankers and NI = Noninoculated controls.

had significantly greater lesion sizes than the burn wounds on the noninoculated controls. The means of three experiments are presented as one figure (fig. 3B). Inoculation with 13,000 conidia/10 µl droplet, an intermediate level of inoculum, resulted in consistent infection. This concentration of spore suspension was used in the remainder of the leaf disc experiments.

Effect of temperature on lesion size.—Two experiments were done to test the effect of temperature on size of lesions on green ash leaf discs inoculated with *G. fraxini*. In both experiments, lesion expansion was greatest at the intermediate temperatures and much less at the extreme high and low temperatures (fig. 3E). Lesions on wounded inoculated discs incubated at 15, 20 and 25 C were significantly larger than the wounds on controls or than any other treatment. Very little lesion expansion occurred at or below 10 C or at 30 C.

Effect of sources of isolates.—The effect that the source of isolates of *G. fraxini* might have on aggressiveness, as determined on inoculated leaf discs, was studied at three temperatures in two experiments. In both experiments, temperature had a significant effect on lesion size with the largest lesions being produced at 22 C (fig. 3F). Lesions developed at all three temperatures, 16, 22 and 28 C. Lesions expanded significantly beyond the burn wound at all temperatures. No significant interaction between temperature and source of isolate was detected. Comparing lesion size produced by isolates from several sources within temperatures, generally the sources did not differ.

Discussion

The role of injuries that occur on ash leaves in nature needs to be investigated because only leaf discs that were wounded and inoculated became infected. Wounding prior to inoculation has been used to study the infection of oak and sycamore leaves by anthracnose fungi (12). Isolations made from leaf spot and leaf blotch symptoms adjacent to insect feeding wounds frequently resulted in a high percent of recovery of *G. fraxini* (16). The defense mechanisms of the host plant could be breached as a result of insect feeding injury. This provides an interpretation of the symptomatology of the disease.

In the phenology studies done on green ash seedlings in the greenhouse, similar amounts of time were required for the completion of a growth flush. Younger tissue of several host/pathogen systems is more susceptible to disease (15). The experiments reported here showed that leaf discs removed from younger green ash leaves were more susceptible to infection by the ash anthracnose fungus than discs from older leaves. This is just the opposite of the situation in walnut anthracnose where older leaves were more susceptible (4). Greater susceptibility of newly emerging leaves of green ash may partially explain why infection occurs primarily in May and June in North Dakota.

The purpose of the experiments that tested leaves of different ages was not to determine susceptibility of leave of certain ages to infection as it occurs in nature but rather to define an optimum age for further in vitro testing; however, these experiments did give an indication of the effect of leaf age on innate susceptibility.

In the first and second experiments on leaf age, the greatest amount of lesion development was observed on discs from 10-day old leaves. In the third and fourth experiments on leaf age, discs from 20-day old leaves had the largest lesions. Ten- and fifteen-day old leaves had leaflets of small width which limited the amount of material available for use as 18 mm discs. The 20-day-old leaf age was chosen as the best compromise between susceptible material and the amount of material available for use. If smaller discs are chosen, younger leaves might provide adequate material but handling might be more difficult.

A comparison of temperature effects on size of lesions on inoculated green ash leaf discs shows that the temperature optimum was between 20 and 25 C (16). The temperature range for infection of leaf discs coincided with previous research done on spore germination and mycelial growth (13,16,18,26).

In several experiments, lesions with diffuse margins were observed on discs incubated in the dark at 20 to 25 C. Black (2) reported that walnut anthracnose also was most severe on leaves maintained under low light intensities.

Snyder's (23) greenhouse and laboratory techniques for infection of ash by *G. fraxini* required relatively long periods of time to evaluate infection and the authors were unable to reproduce those results reliably. The results reported here indicate that the excised leaf disc inoculation method can successfully be applied to study the infection of green ash by *G. fraxini*. The experiments that explored the effect of inoculum concentration were done not to define an optimum concentration but to determine a rate for consistent infection of leaf discs.

Value of method.—The agar leaf disc testing method has value as a research tool to study the life history of anthracnose diseases of temperate trees. The excised leaf disc method can be successfully utilized to study the infection of green ash by *G. fraxini*. It provides a method of investigation that bridges the gap between field or greenhouse methods and tissue culture methods. In vitro methods were used to duplicate many of the symptoms and signs of the disease that were observed in nature.

The advantages of this technique are that standard research facilities, laboratory equipment and supplies are all that are needed. Lesions on inoculated discs are easily evaluated in less than 10 days. In further studies not reported here, Redlin (16) compared susceptibility of three *Fraxinus* species using the leaf disc technique. This method will be useful to explore inherent differences in susceptibility among clones, cultivars, or seed sources especially since large numbers of individual trees can be screened rapidly and in a limited amount of space.

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Literature Cited

1. Beceresçu, D. 1975. Metoda pentru test area rapida a patogenitatii la izolatele monosporale ale ciupercii *Venturia inaequalis*. Analele Institului de Cercetari pentru Portectia Plantelor 1973, II:89-99.

2. Black, W. M. 1977. Ecology and epidemiology of walnut anthracnose. Ph.D. dissertation, Univ. of Illinois, Urbana. 83 p.

3. Chee, K. H., Darmono, T.W., Santos, A.F. dos. 1986. Laboratory screening of fungicides using cellulose film and leaf discs against South American leaf blight pathogen, *Microcyclus ulei*. J. Nat. Rubber Res. 1:98-103.

4. Cline, S. D., Neely, D. 1984. Relationship between juvenile-leaf resistance to anthracnose and the presence of juglone and hydrojuglone glucoside in black walnut. Phytopathology 74:185-188.

5. Currah, L., Maude, R.B. 1984. Laboratory tests for leaf resistance to *Botrytis squamosa* in onions. Ann. Appl. Biol. 105:277-283.

6. Davies, R. M., Heale, J.B. 1985. *Botrytis cinerea* in stored cabbage: the use of germ tube growth on leaf discs as an indicator of potential head rot. Plant Pathology 34:408-414.

7. Dhinga, O. D., Sinclair, J.B. 1985. Basic Plant Pathology Methods. CRC Press, Boca Raton, Florida, 355 p.

8. Eenink, A. H., DeJong, C.J. 1982. Partial resistance in lettuce to downy mildew (*Bremia lactucae*). 3. Correspondence between resistance levels of cotyledons and leaf discs. Euphytica 31:761-770.

9. Eskes, A. B. 1982. The use of leaf disc inoculations in assessing resistance to coffee leaf rust (*Hemileia vastatix*). Neth. J. Pl. Path. 88:127-141.

10. Hilebrand, D. C., Schroth, M.N. 1964. Arbutin-hydroquinone complex in pear as a factor in fireblight development. Phytopathology 54:640-645.

11. Kapooria, R. G., Tjallingii, F. 1969. A new method for inoculation of lettuce with *Bremia lactucae*. Neth. J. Pl. Path. 75:224-226.

12. Neely, D., Himelick, E.B. 1967. Characteristics and nomenclature of the oak anthracnose fungus. Phytopathology 57:1230-1236.

13. Ogawa, J., Bose, E.M., Manji, B.T., Petersen, L.J. 1977. Life cycle and control of Modesto tree anthracnose. Plant Dis. Rep. 61:792-796.

14. Ostry, M. E., Skilling, D.D. 1988. Somatic variation in resistance of *Populus* to *Septoria musiva*. Plant Dis. 72:724-727.

15. Populer, C. 1978. Changes in host susceptibility with time. p. 239-262. In J. G. Horsfall & E. B. Cowling (eds). Plant Disease: An Advanced Treatise. Vol. 2, Academic Press, New York. 436 p.

16. Redlin, S. C. 1988. The biology and taxonomy of Gnomoniella fraxini; cause of ash anthracnose. Ph.D. dissertation. North Dakota State University, Fargo. 201 p.

17. Redlin, S. C., Stack, R.W. 1985. A laboratory technique to evaluate infection of green ash by *Gloeosporium aridum*. Proc. North Dakota Acad. Sci. 39:36.

18. Redlin, S. C., Stack, R.W. 1986. Effect of temperature on germination of conidia of *Gloeosporium aridum*. Proc. North Dakota Acad. Sci. 40:63.

19. Redlin, S. C., Stack, R.W. 1988. *Gnomoniella fraxini* sp. nov., teleomorph of the ash anthracnose fungus and its connection to *Discula fraxinea* comb. nov. Mycotaxon 32:175-198.

20. Santamour, F. S., Jr., McArdle, A.J. 1983. Checklist of cultivars of North American ash (*Fraxinus*) psecies. J. Arbor. 9:271-276.

21. Shain, L., Cornelius, P.L. 1979. Quantitative inoculation of eastern cottonwood leaf tissue with *Melampsora medusae* under controlled conditions. Phytopathology 69:301-304.

22. Sinclair, W. A., Lyon, H.H., Johnson. W.T. 1987. Diseases of Trees and Shrubs. Cornell Univ. Press, Ithaca, NY. 574 p.

23. Snyder, T. E. 1983. Occurrence and pathogenicity of *Gloeosporium aridum* on green ash. M. S. thesis. North Dakota State University, Fargo. 70 p.

24. Snyder, T. E., Stack, R.W. 1983. Occurrence of *Gloeosporium aridum* on green ash at different times of the year. Proc. North Dakota Acad. Sci. 37:95.

25. Spiers, A. G. 1978. An agar leaf-disc technique for screening poplars for resistance to *Marssonina*. Plant Dis. Rep. 62:144-147.

26. Stack, R. W., Snyder, T.E., Redlin, S.C. 1990. Occurrence and pathogenicity of *Gnomoniella fraxini*, cause of ash anthracnose. p. 130-136. In: W. Merrill and M.E.Ostry (eds.). Recent Research on Foliage Diseases. USDA For. Serv. Gen. Tech. Rep. WO-56, 145 p.

27. Steele, R. G. D., Torrie, J.H. 1980. Principles and Procedures of Statistics - A Biometrical Approach. 2nd ed.. McGraw-Hill Book Co., New York. 633 p.

28. Yarwood, C. E. 1946. Detached leaf culture. Bot. Rev. 12:1-56.